The Translational and Molecular Imaging Institute presents:

The 2nd Annual TMII Symposium-2011





Program Book



The New York Academy of Medicine 1216 5th Ave

May 27th, 2011



Content

Message from the Institute Director and the Dean of Mount Sinai	2
Program	3
Biographies from invited speakers	
Zahi A. Fayad, Ph.D.	5
Dennis S. Charney, M.D.	7
Robert S. Langer, Sc.D.	9
Denis B. Buxton, Ph.D.	11
John V. Frangioni, M.D., Ph.D.	12
Jeff W.M. Bulte, Ph.D.	14
Kelvin Lim, M.D.	15
Gregory Sorensen, M.D.	16
Burton Draver, M.D	17
Translational and Molecular Imaging Institute	
Mission of the Translational and Molecular Imaging Institute	19
Resources of the Translational and Molecular Imaging Institute	21
Cardiovascular Imaging Program	23
Neuro Imaging Program	24
Nanomedicine Program	25
Program of Excellence in Nanotechnology (PEN) NIH/NHLBI	26
Cell Tracking Core	27
Image Analysis Core	28
Abstracts selected for oral presentation	
Abstract selected for Cardiovascular Imaging category (Duivenvoorden)	
Abstract selected for Neuro Imaging category (Hill)	
Abstract selected for Nanomedicine category (Shapiro)	
Abstract selected for Neuro Imaging category (Berlin)	
Abstracts selected for poster presentation, Cardiovascular Imaging category	
Abstracts selected for poster presentation, Neuro Imaging category	
Abstracts selected for poster presentation, Nanomedicine category	
Abstracts selected for poster presentation, Cancer Imaging category	



Message from the Translational Molecular Imaging Institute Director and the Dean of the Mount Sinai School Of Medicine

It is with great enthusiasm and pride that we introduce the 2nd Annual Translational Molecular Imaging Institute (TMII) Symposium.

The TMII symposium is intended to offer some snapshots of most current translational imaging research at Mount Sinai and other institutions in and outside the New York metropolitan area.

The TMII team and the Mount Sinai School of Medicine have carefully worked to organize this symposium, and we look forward to familiarizing you with our Institute and its research endeavors. We encourage you to review the Program Book, which provides abstracts and the program summary. In addition, it includes information about the speakers, activities, facilities and faculty members at our Institute and Mount Sinai's efforts in translational research.

Welcome to New York and Mount Sinai.



Zahi A. Fayad, Ph.D.

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TMII director Professor of Radiology Professor of Medicine



Dennis S. Charney, MD

S Chang no

Anne and Joel Ehrenkranz Dean, Mount Sinai School of Medicine Executive Vice President for Academic Affairs, The Mount Sinai Medical Center

	T	Fhe 2 nd Annual TMII Symposium – 2011											Friday, May 27, 2011												
Affiliation		Director, Translational Molecular Imaging Institute	Dean, Mount Sinai School of Medicine	Massachusetts Institute of Technology	hi A. Fayad, PhD	National Institute of Health/NHLBI		Mount Sinai School of Medicine		ichir Taouli, MD) Beth Israel Deaconess Medical Center ons and Contrast Agents for Image-Guided Surgery"	New York University School of Medicine	bo, PhD & Venkatesh Mani, PhD			llem Mulder, PhD	The Johns Hopkins University School of Medicine inic"	Yale School of Medicine		PhD & Matilde Inglese, MD	University of Minnesota	Mount Sinai School of Medicine	Massachusetts General Hospital	Chair of Radiology, Mount Sinai School of Medicine	
Speaker		Zahi A. Fayad, PhD	Dennis Charney, MD	Robert S. Langer, ScD	Session I - Moderator: Zal	Denis B. Buxton, PhD	ular Imaging"	Raphael Duivenvoorden		Session II - Moderator: Ba	John V. Frangioni, MD, PhD I Fluorescence Imagina Syste	Lindsay K. Hill	acilitators: Karen Briley-Sae			Session IV - Moderator: Wi	Jeff W.M. Bulte, PhD Cell Therapy: Entering the Cl	Erik M. Shapiro		- Moderators: Cheuk Tang	Kelvin Lim, MD for the Study of Brain Disord	Heather A. Berlin	A. Gregory Sorensen, MD /PET, 7T and beyond"	Burton P. Drayer, MD	Zahi A. Fayad, PhD
Category	Registration/Breakfast	Opening remarks	Opening remarks	Keynote speaker ne"		Translational research	of Cardiovascular Molec	Cardiac imaging	Break		Cancer imaging nslation of Near-Infrare	Neuro imaging	Session III - F	Poster viewing	Lunch		Cell therapy al Cellular Imaging and	Cell tracking	Break	Session V	Neuro imaging ity Magnetic Resonance	Neuro imaging	Neuro imaging es in Neuroimaging: MR	Closing remarks	Adjournment
Time	7:00-8:00	8:00-8:15	8:15-8:30	8:30-9:30 "Nanomedici		9:30-10:15	"Translation	10:15-10:30	10:30-11:00		11:00-11:45 "Clinical Trai	11:45-12:00		12:00-1:30	12:00-1:30		1:30-2:15 "Translation"	2:15-2:30	2:30-3:00		3:00-3:45 "Multimodali	3:45-4:00	4:00-4:45 "New advanc	4:45-5:00	5:00



The Translational and Molecular Imaging Institute presents: The 2nd Annual TMII Symposium-2011

Biographies of invited speakers





Zahi A. Fayad, Ph.D., FAHA, FACC

Professor of Radiology, and Medicine (Cardiology) Director of the Translational and Molecular Imaging Institute (TMII) Mount Sinai School of Medicine



Biography

Dr. Fayad serves as professor of Radiology and Medicine (Cardiology) at the Mount Sinai School of Medicine. He is the Director of the Translational and Molecular Imaging Institute; Director and Founder of the Eva and Morris Feld Imaging Science Laboratories; and Director of Cardiovascular Imaging Research at the Mount Sinai School of Medicine and Mount Sinai Medical Center. He has made many contributions to the clinical and preclinical research cardiovascular sciences (MRI, multimodality imaging, and molecular imaging) through his seminal work on atherosclerosis imaging. Dr. Fayad's is one of the world's leader in the development and use of multimodality cardiovascular imaging including, Cardiovascular Magnetic Resonance (MR), computed tomography (CT), and positron emission tomography (PET), as well as molecular imaging and nanomedicine to study and treat cardiovascular disease. His focus in the past 14 years at Mount Sinai has been on the noninvasive assessment and understanding of atherosclerosis (Sanz and Fayad Nature 2008; 451:953-957). He holds 12 US and Worldwide patents in the field of imaging. He has authored more than 250 peer-reviewed publications, 50 book chapters, and over 400 meeting presentations. He is currently the Principal Investigator of four federal grants funded by the National Institutes of Health's National Heart, Lung and Blood Institute and National institute of Biomedical Imaging and Bioengineering with a recent large award from NHLBI to support the Program of Excellence in Nanotechnology. In addition, he serves as Principal Investigator of the Imaging Core of the Mount Sinai National Institute of Health (NIH)/Clinical and Translational Science Awards (CTSA). Dr. Fayad had his trainings at the Johns Hopkins University and at the University of Pennsylvania. From 1996 to 1997 he was junior faculty in the Department of Radiology and the University of Pennsylvania. In 1997 he joined the faculty at Mount Sinai School of Medicine. Dr. Fayad is past-deputy Editor of Magnetic Resonance in Medicine (MRM), past-president of the Society of Atherosclerosis and Prevention (SAIP), fellow of the American Heart Association (AHA) where he served on the National Research Committee and on the Council on Cardiovascular Radiology and Intervention (CVRI). He is also a fellow of the American College of Cardiology (ACC), where he served on the Cardiovascular Collaborative Imaging (CCI) Committee. He is member of the NIH's National Lung, and Blood Institute (NHLBI) Cardiovascular Strategic Planning Working Group on Vascular Disease and Hypertension. He is a member of the Foundation of the NIH (FNIH) Biomarkers Consortium. Dr. Fayad is Associate Editor for the Journal of the American College of Cardiology Imaging (JACC Imaging) and Consulting Editor for Arteriosclerosis Thrombosis and Vascular Biology (ATVB). Dr. Fayad is on the editorial boards of Journal of Cardiovascular Magnetic Resonance (JCMR), Nature Reviews Cardiology, Atherosclerosis, and Cancer Nanotechnology. He often serves as guest editor for several prestigious journals in the fields of imaging, vascular biology, cardiology and radiology. He participates regularly to the AHA/ACC writing groups. He is a member of the Medical Imaging (MEDI) study section and ad-hoc member on numerous other study sections including those from NIH and the National Academy of Science. He is a member of the New York University Program in Computational Biology. He is a past member of the



board of trustees of the Society of Cardiovascular Magnetic Resonance (SCMR) and he is also a past member of the Scientific Program Committee of the International Society of Magnetic Resonance in Medicine (ISMRM). He also serves on the boards of several national and international scientific boards, committees and foundations. Dr. Fayad is the recipient of multiple prestigious awards. In 2007 he was given the John Paul II Medal from Krakow, Poland in recognition for the potential of his work on humankind. As a teacher and mentor, Dr. Fayad has been also extremely successful. He has trained over 30 postdoctoral fellows, clinical fellows and students. His trainees have received major awards, fellowships, and positions in academia and industry. In 2008 he received the Outstanding Teacher Award from the International Society of Magnetic Resonance in Medicine (ISMRM) for his teaching on cardiovascular imaging and molecular imaging. Recently, in 2009 he was awarded the title of Honorary Professor in Nanomedicine at Aarhus University in Denmark. He is married to Monique P. Fayad, MBA and is the proud father of Chloé (9 year old) and Christophe (5 year old) and after spending seven years in Manhattan now lives and sails in Larchmont, NY.



Dennis S. Charney, M.D.

Professor of Psychiatry, Neuroscience, and Pharmacology and Systems Therapeutics Dean, Mount Sinai School of Medicine



Biography

The arrival of Dennis S. Charney, MD, at Mount Sinai in 2004 signaled a new era of innovation in research, education, and clinical care. Since joining the faculty, he has established a culture of excellence that has elevated Mount Sinai School of Medicine — an institution founded in 1968 — to among the top medical schools in the nation. With an emphasis on translational research, Dr. Charney has accelerated the pace of change at Mount Sinai, streamlined collaboration across disciplines, and facilitated the integration of research, clinical care, and educational innovation. These efforts have produced remarkable results. Mount Sinai School of Medicine now ranks 18th in National Institutes of Health (NIH) funding — an increase from 25th in 2004 — and in the past five years, its position in U.S. News & World Report has risen from 32 to 18. No other medical school in America has achieved this degree of improvement in such a brief period. Early in his tenure at Mount Sinai, Dr. Charney led the creation of the School of Medicine's Strategic Plan, an organizational restructuring that included the creation of 14 interdisciplinary research institutes. As a medical school embedded in a hospital, Mount Sinai has always integrated research and clinical medicine. These institutes — chosen in the areas where Mount Sinai can truly excel — embody the institution's mission as a leader in basic and clinical research. A leading investigator on neurobiology and the treatment of mood and anxiety disorders, Dr. Charney has made fundamental contributions to the understanding of neural circuits and neurochemistry related to human anxiety, fear, and mood. He has pioneered research related to the psychobiological mechanisms of human resilience to stress. In addition, his research team has made major contributions to the discovery of novel and more effective treatments for mood and anxiety disorders.

Dr. Charney's distinguished career as a researcher and educator began in 1981 at Yale University School of Medicine, where, within nine years, he rose from Assistant Professor to Professor of Psychiatry, a position he held from 1990 to 2000. While at Yale, Dr. Charney chaired the National Institute of Mental Health (NIMH) Board of Scientific Counselors, which advises the institute's director on intramural research programs.

After nearly two decades at Yale, NIMH recruited Dr. Charney to lead the Mood and Anxiety Disorder Research Program — one of the largest programs of its kind in the world — and



the Experimental Therapeutics and Pathophysiology Branch. That year he was also elected to the Institute of Medicine of the National Academy of Sciences. His scientific research has been honored by every major award in his field.

Dr. Charney remained at NIMH until he was recruited to Mount Sinai in 2004 as Dean of Research. Two years later, he was appointed Dean for Academic and Scientific Affairs for Mount Sinai School of Medicine and Senior Vice President for Health Sciences of The Mount Sinai Medical Center. In 2007, Dr. Charney became the Dean of Mount Sinai School of Medicine and Executive Vice President for Academic Affairs of the Medical Center. The following year, he was named the Anne and Joel Ehrenkranz Dean of Mount Sinai School of Medicine.

A prolific author, Dr. Charney has written more than 700 publications, including groundbreaking scientific papers, chapters, and books. He has authored a dozen books, including Neurobiology of Mental Illness (Oxford University Press, USA, Third Edition, 2009); The Peace of Mind Prescription: An Authoritative Guide to Finding the Most Effective Treatment for Anxiety and Depression (Houghton Mifflin Harcourt, 2004); and The Physicians Guide to Depression and Bipolar Disorders (McGraw-Hill Professional, 2006). In 2011, Dr. Charney plans to publish his 13th book, which addresses emotional resilience.



Robert S. Langer, Sc.D. David H. Koch Institute Professor Massachusetts Institute of Technology



Biography

Robert S. Langer is the David H. Koch Institute Professor (there are 14 Institute Professors at MIT; being an Institute Professor is the highest honor that can be awarded to a faculty member). Dr. Langer has written nearly 1,120 articles. He also has approximately 790 issued and pending patents worldwide. Dr. Langer's patents have been licensed or sublicensed to over 220 pharmaceutical, chemical, biotechnology and medical device companies. He is the most cited engineer in history.

He served as a member of the United States Food and Drug Administration's SCIENCE Board, the FDA's highest advisory board, from 1995 -- 2002 and as its Chairman from 1999-2002.

Dr. Langer has received over 180 major awards including the 2006 United States National Medal of Science; the Charles Stark Draper Prize, considered the equivalent of the Nobel Prize for engineers and the 2008 Millennium Prize, the world's largest technology prize. He is the also the only engineer to receive the Gairdner Foundation International Award; 72 recipients of this award have subsequently received a Nobel Prize. Among numerous other awards Langer has received are the Dickson Prize for Science (2002), Heinz Award for Technology, Economy and Employment (2003), the Harvey Prize (2003), the John Fritz Award (2003) (given previously to inventors such as Thomas Edison and Orville Wright), the General Motors Kettering Prize for Cancer Research (2004), the Dan David Prize in Materials Science (2005), the Albany Medical Center Prize in Medicine and Biomedical Research (2005), the largest prize in the U.S. for medical research, induction into the National Inventors Hall of Fame (2006), the Max Planck Research Award (2008) and the Prince of Asturias Award for Technical and Scientific Research (2008). In 1998, he received the Lemelson-MIT prize, the world's largest prize for invention for being "one of history's most prolific inventors in medicine." In 1989 Dr. Langer was elected to the Institute of Medicine of the National Academy of Sciences, and in 1992 he was elected to both the National Academy of Engineering and to the National Academy of Sciences. He is one of very few people ever elected to all three United States National Academies and the youngest in history (at age 43) to ever receive this distinction.

Forbes Magazine (1999) and Bio World (1990) have named Dr. Langer as one of the 25 most important individuals in biotechnology in the world. Discover Magazine (2002) named him as one of the 20 most important people in this area. Forbes Magazine (2002)



selected Dr. Langer as one of the 15 innovators world wide who will reinvent our future. Time Magazine and CNN (2001) named Dr. Langer as one of the 100 most important people in America and one of the 18 top people in science or medicine in America (America's Best). Parade Magazine (2004) selected Dr. Langer as one of 6 "Heroes whose research may save your life." Dr. Langer has received honorary doctorates from Harvard University, the Mt. Sinai School of Medicine, Yale University, the ETH (Switzerland), the Technion (Israel), the Hebrew University of Jerusalem (Israel), the Universite Catholique de Louvain (Belgium), Rensselaer Polytechnic Institute, Willamette University, the University of Liverpool (England), Bates College, the University of Nottingham (England), Albany Medical College, Pennsylvania State University, Northwestern University, Uppsala University (Sweden) and the University of California – San Francisco Medal. He received his Bachelor's Degree from Cornell University in 1970 and his Sc.D. from the Massachusetts Institute of Technology in 1974, both in Chemical Engineering.

Abstract: "Nanomedicine"

There are numerous new nanotechnology based systems being developed that may impact the future of medicine. For example, new drug delivery technologies including microparticles, nanoparticles and nanotechnology promise to create new treatments for cancer, heart disease and other illnesses. Nanotechnology may also be useful in delivering DNA and siRNA. Approaches involving polymers, microchips, and lipids will be discussed. Furthermore, new types of biomaterials and biosensors utilizing nanotechnology will be examined.



Denis B. Buxton, Ph.D.

Associate Director of the Basic and Early Translational Research Program DCVS, National Heart, Lung, and Blood Institute (NHLBI), Bethesda M



Biography

Dr. Buxton is the Associate Director of the Basic and Early Translational Research Program at the National Heart, Lung and Blood Institute (NHLBI). The program includes two branches, Advanced Technologies and Surgery, and Vascular Biology and Hypertension. After obtaining his PhD in Biochemistry at University College, London, he went to the University of Texas Health Science Center in San Antonio working on substrate metabolism. He then joined UCLA, where as an associate professor of pharmacology he studied cardiac ischemia and reperfusion using positron emission tomography. He came to NHLBI via the intramural program, where he worked on signal transduction and nonmuscle myosins.

Abstract: "Translation of Cardiovascular Molecular Imaging"

The last 20 years have seen a rapid growth in the development of molecular imaging probes and technology, and in their application to cardiovascular disease. However, the clinical application of molecular imaging to cardiovascular diseases has been limited so far. The NHLBI recently held a working group to address the state of the field, to assess clinical needs, and to explore barriers to the translation of cardiovascular molecular imaging and how they might be overcome. This talk will focus on unmet clinical needs, preclinical and early clinical studies that show promise for meeting these needs, and ways that the clinical application of molecular imaging to cardiovascular diseases might be accelerated.



John V. Frangioni, M.D., Ph.D.

Associate Professor of Medicine and Associate Professor of Radiology Beth Israel Deaconess Medical Center and Harvard Medical School Co-Director, Center for Molecular Imaging, Beth Israel Deaconess Medical Center Co-Director, Longwood Small Animal Imaging Facility, Beth Israel Deaconess Medical Center Editorial Board, Molecular Imaging



Biography

Dr. Frangioni is an internationally renowned expert in molecular imaging. His laboratory focuses on the development of novel medical devices and disease-specific contrast agents for diagnosing and treating cancer. Dr. Frangioni is inventor of the FLARE[™] and mini-FLARE[™] intraoperative near-infrared fluorescence imaging systems, which use invisible light to find tumors, blood vessels, nodes, and nerves during surgery. His laboratory has also developed numerous cancer- and disease-specific contrast agents for use with FLARE[™]. The FLARE[™] and mini-FLARE[™] systems are currently being used in clinical trials for breast cancer sentinel lymph node mapping, lung cancer sentinel lymph node mapping, melanoma sentinel lymph node mapping, and breast cancer reconstructive surgery. Dr. Frangioni is in the process of constructing the first Translational Cancer Imaging Facility at the Beth Israel Deaconess Medical Center, and a major focus of his laboratory is the development of cancer-specific radiotracers for positron emission tomography.

Dr. Frangioni is founder and unpaid director of The FLARE Foundation, a non-profit organization focused on promoting the dissemination of medical imaging technology for research and clinical use.

Dr. Frangioni has been the Principal Investigator of twelve peer-reviewed research grants from the National Institutes of Health totaling over \$42M dollars. He has published over 100 peer-reviewed publications and is committed to sharing the results of his NIH-funded research with other academic investigators. A data sharing repository can be found on his website (www.frangionilab.org). His research has been featured in the Boston Globe, Popular Science, ABC News Science Central, Canadian Public Radio, and Bavarian Public Television.

Dr. Frangioni is an invited reviewer for numerous international foundations and 27 highimpact peer reviewed journals and is also on the Editorial Board of Molecular Imaging. He is a frequent invited speaker or keynote speaker at national and international meetings and is also a dedicated teacher, having trained over 50 post-doctoral fellows, visiting professors, research associates, and students.



Dr. Frangioni is the recipient of several awards including Mentor of the Year at Harvard Medical School and the Edward M. Kennedy Award for Healthcare Innovation, and he has been inducted into the American Society for Clinical Investigation. He is also a Visiting Professor of the Royal Netherlands Academy of Arts and Sciences at the Leiden University Medical Center.

Dr. Frangioni received his undergraduate degree in Engineering Sciences from Harvard College. He received his M.D. from Harvard Medical School and the Massachusetts Institute of Technology's Health Science and Technology (HST) Program, and his Ph.D. in Cellular and Molecular Physiology from Harvard Graduate School of Arts and Sciences. He completed an internal medicine residency at Brigham and Women's Hospital and a medical oncology fellowship at Beth Israel Deaconess Medical Center. He has been board certified in internal medicine and medical oncology, and is an Attending Physician in the Division of Hematology/Oncology at the Beth Israel Deaconess Medical Center.

Abstract: "Clinical Translation of Near-Infrared Fluorescence Imaging Systems and Contrast Agents for Image-Guided Surgery"

Human surgery is currently performed "blindly," without visual highlighting of tissue that needs to be resected, such as tumors, and tissue that needs to be avoided, such as nerves and blood vessels. For this talk I will discuss the preclinical and clinical translation activities in image-guided surgery performed over the last decade at the Center for Molecular Imaging of Harvard Medical School's BIDMC. I will also discuss the key engineering, chemistry, and regulatory issues that will hopefully help inform other optical imaging technologies. The Fluorescence-Assisted Resection and Exploration (FLARE[™]) surgical imaging system was designed to image two independent channels of invisible near-infrared (NIR) fluorescent light, centered at 700 nm and 800 nm, respectively, simultaneously and in real-time with color video. FLARE[™] and its miniaturized version, mini-FLARE[™], have already been translated to the clinic, and to date have been used to image hundreds of patients in clinical studies around the world. Examples from sentinel lymph node mapping of cancer, NIR fluorescence angiography for plastic and reconstructive surgery, and tumorspecific imaging will be presented. NIR fluorescent contrast agents for use with FLARE™ span the spectrum from organic small molecules to inorganic/organic hybrid nanoparticles. The kev chemical design criteria for NIR fluorescent contrast agents will be described, and a new class of zwitterionic NIR fluorophores, which are the subject of impending first-inhuman clinical trials, will be described. Multiple imaging systems and contrast agents for image-guided human surgery using NIR fluorescent light are now available and are the subject of intense preclinical and clinical investigation. The next decade will likely clarify whether or not intraoperative NIR fluorescence image-guidance is capable of changing patient management and thus entering into the surgeon's armamentarium.



Jeff W.M. Bulte, Ph.D.

Professor of Radiology, Biomedical Engineering, and Chemical & Biomolecular Engineering Director of the Cellular Imaging Section

Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, Department of Biomedical Engineering, and Department of Chemical & Biomolecular Engineering, Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA



Biography

Jeff W.M. Bulte is a Professor of Radiology at the Johns Hopkins University School of Medicine, with joint appointments in Biomedical Engineering and Chemical & Biomolecular Engineering. He serves as the Director of the Cellular Imaging Section in the Institute for Cell Engineering. Previously, he was a scientist at the National Institutes of Health, and obtained his Ph.D. degree Summa Cum Laude from the University in Groningen in The Netherlands.

Abstract: "Translational Cellular Imaging and Cell Therapy: Entering the Clinic"

The purpose of this abstract is to illustrate how translational cellular imaging is expected to play a key role in evaluating the outcome of many cell therapeutic clinical trials. In order to facilitate and implement the translation of novel experimental cell therapies into the clinic, one needs to be able to monitor the cellular biodistribution non-invasively following administration. Among the different clinically used cellular imaging techniques, 111In oxine scintigraphy is the only FDA-approved method and has been primarily for imaging of infection and inflammation. Cellular magnetic resonance (MR) imaging, with its superior spatial resolution and excellent soft tissue anatomical detail, is emerging as the technique of choice to monitor in real-time image-guided cell delivery, immediate engraftment, and short-term homing. Up until last year, 6 clinical studies have been published, all using superparamagnetic iron oxide nanoparticles or SPIOs in an off-label fashion. SPIOs are clinically approved and create strong local magnetic field disturbances that spoil the MR signal leading to hypo- or hyperintense contrast. A major setback is that the particles that were being used have been taken off the market, as their primary, FDA-approved indication (liver imaging of Kupffer cells) did not live up to its promise. However, several companies have started production runs of novel particles, that possibly can also be used for magnetic particle imaging (MPI). Several other cellular imaging techniques are available, some of which are based on reporter genes, e.g. firefly luciferase for bioluminescent imaging, and herpes simplex virus thymidine kinase for positron emission tomography imaging. While the former cannot be used clinically because of physico-optical constraints, the latter has now also entered the clinic.



Kelvin Lim, M.D.

Professor of Psychiatry Center for Magnetic Resonance Research, University of Minnesota, Twin Cities



Biography

Dr. Lim holds the Drs. T. J. and Ella M. Arneson Land-Grant Chair in Human Behavior at the University of Minnesota where he is a Professor of Psychiatry and Vice Chair for Research. Dr. Lim began his undergraduate work at the Massachusetts Institute of Technology, completing his Bachelor and MD degrees at Johns Hopkins University. He completed a psychiatry residency and fellowship in neuroimaging at Stanford University where he also served on the faculty in the Department of Psychiatry. He joined the University of Minnesota in 2001. Dr. Lim's research interest is the use of innovative magnetic resonance imaging techniques to study brain disorders. His primary focus has been the use of multiple modalities available with magnetic resonance to characterize the brain in schizophrenia, aging, traumatic brain injury and cocaine dependence.

Abstract: "Multimodality Magnetic Resonance for the Study of Brain Disorders"

Magnetic resonance (nuclear magnetic resonance) was first applied to elucidating the chemical structure of liquids and solids. It was not until the 1980s that commercial magnetic resonance imaging scanners became available, providing diagnostic anatomical images with unparalleled tissue contrast of the brain and other organs. Since then, magnetic resonance has evolved into a very flexible imaging method. An advantage of MR for research studies is the lack of ionizing radiation. MR has an expanding list of modalities, which can provide unique information, all with the same scanner in the same imaging session. For example, MR can provide information about anatomy, chemistry, diffusion, perfusion and function, all with the same equipment. The possibility of being able to acquire multiple types of information from the same subject provides a powerful tool for studying brain disorders.



A. Gregory Sorensen, M.D.

Professor of Radiology and Health Sciences & Technology Harvard Medical School Massachusetts General Hospital



Biography

Dr. Sorensen serves as Professor of Radiology and Health Sciences and Technology at Harvard Medical School and at the Massachusetts Institute of Technology, and is a visiting professor of neuroradiology at Oxford University. He is the Co-Director of the Martinos Center for Biomedical Imaging at Massachusetts General Hospital, the largest and most sophisticated imaging center in the world. His research interests include development and use of advanced imaging methods to investigate human illness, particularly neurological diseases such as stroke and brain cancer. He is a funded NIH investigator and leads a number of clinical trials investigating the diagnostic power of MRI, PET and most recently, combined simultaneous MR-PET.

Abstract: " New advances in Neuroimaging: MR/PET, 7T and beyond "

Neuropsychiatric illnesses constitute the single largest category of the global burden of disease. Despite this impact, imaging has yet to have a meaningful role in many neurological diseases. Fortunately, new advances in imaging technology are enabling visualization of structure, function, and pathophysiology at a new level. This presentation will review some of the advances that appear to have the most promise for moving the field ahead, including 7 Tesla MRI, combined MR-PET scanning, and other new methods.



Burton Drayer, M.D., F.A.C.R.

Professor of Radiology Chair of the Department of Radiology, Mount Sinai School of Medicine



Biography

Burton Paul Drayer, MD is currently the Dr. Charles M. and Marilyn Newman Professor and Chairman of the Department of Radiology (1995-present) Mount Sinai School of Medicine and the Executive Vice President for Risk at The Mount Sinai Medical Center. Additionally, from 2003 to 2008, Dr. Drayer served as President of The Mount Sinai Hospital. He completed his internship and Neurology residency at the University of Vermont and then a Radiology residency and Neuroradiology fellowship at the University of Pittsburgh Health Center. He is Board certified in both Neurology and Radiology and a fellow of both the American College of Radiology and the American Academy of Neurology.

Dr. Drayer served as Associate Professor and Professor of Radiology at Duke University from 1979 to 1986 where he was also Director of Neuroradiology. In 1986, he joined the Barrow Neurological Institute as Director of Magnetic Resonance Imaging and Research. Internationally known for his CT and MRI research on the aging brain and neurodegenerative disorders, brain infarction, multiple sclerosis, and physiological and functional brain imaging, Dr. Drayer has written over 200 publications as well as multiple book chapters. He was the first to describe metrizamide encephalopathy, nonradioactive xenon enhanced CT for measuring rCBF (ASNR Cornelius G. Dyke Award 1977) and the normal and abnormal distribution of brain iron using MRI. He also popularized carotid and intracranial MRA and educated a generation of physicians in the efficient clinical use of brain and spine MRI. He has been on numerous editorial boards and was the editor of Neuroimaging Clinics of North America from 1990 to 2005.

Dr. Drayer was elected President of the ASNR in 1996, was the inaugural Chairman of its Research Foundation, and was awarded the ASNR Gold Medal in 2011. In 2003, Dr. Drayer was elected to the Board of Directors of the RSNA and in 2009 ascended to Chairman of the Board, 2010 President elect, and 2011 RSNA President. He also presently serves on the Board of Chancellors of the ACR and the Board of Trustees of the RSNA Research and Education Foundation, is a past-President of the New York Roentgen Ray Society, and has served on numerous national advisory boards for multiple sclerosis, stroke, and Alzheimer's disease.

Translational and Molecular Imaging Institute







Mission of the Translational and Molecular Imaging Institute



Zahi A. Fayad, Ph.D. Director, TMII



Juan Gilberto Aguinaldo, M.D. Financial Manager, TMII



Inna Gurewitz, M.P.H. Grants Manager, TMII

The mission of *The Translational and Molecular Imaging Institute (TMII)* is to research, develop, validate and apply a new generation of imaging methodologies in order to advance preclinical and translational research activities. This will result in new methods for drug delivery, as well as in the establishment of new classes of contrast agents which may improve disease detection, disease characterization, clinical diagnosis, treatment and monitoring of therapeutic responses.

The institute aims to promote an array of next-generation, world-class biomedical imaging resources to be used by clinical and translational researchers of the various institutes at Mount Sinai as well as by our collaborators. TMII provides advanced, highly efficient and cost-effective imaging services, and offers expertise for the development and validation of new procedures, encouraging interdisciplinary collaborations that merge the gap between preclinical and clinical research.

One of the key missions of the institute is to educate researchers, postdoctoral fellows, students and technicians about biomedical imaging advances through targeted seminars, research fellowships, publications, established training programs and symposiums such as the one held today.

Currently, TMII is organized in three different Programs and a Core: The Cardiovascular Imaging Program is headed by Zahi A. Fayad, Ph.D.; the Neuroimaging Program by Cheuk Y. Tang, Ph.D., the Nanomedicine Program by Willem J.M. Mulder, Ph.D.; and the Cell Tracking Core is directed by Karen Briley-Saebo, Ph.D.

TMII is at the forefront of advancing the science of imaging and translational research, and facilitates the transformation of clinical medicine.





Center for Science and Medicine

Mount Sinai's new Center for Science and Medicine will combine research and clinical care while providing job opportunities to the local community. NEW YORK, NY

January 3, 2009 /Press Release/

With construction underway on its new Center for Science and Medicine, The Mount Sinai Medical Center is looking forward to significantly expanding its research and treatment programs while providing hundreds of job opportunities to local residents.

Construction and operation of the Center for Science and Medicine at E. 102nd St., between Madison and Fifth Avenues, will provide concrete economic and health benefits to Mount Sinai's neighboring East Harlem community. Mount Sinai is already the leading employer in East Harlem, and nearly one-half of the visits to its outpatient clinics are from Harlem residents.

Health benefits

- Expanded research in cancer, brain, heart disease, asthma, diabetes, hypertension, obesity and other areas of research and clinical care
- More than 200 physicians, nurses, social workers, nutritionists, medical assistants, registration and financial personnel, and other administrative staff to support the Center's outpatient facilities
- 400 new patient visits per day
- More than \$350 million in additional National Institutes of Health funding during the first five years

Economic benefits

- More than 650 permanent new employment opportunities with an annual payroll of nearly \$40 million. The new jobs include 100 faculty members, 100 graduate students, 125 postdoctoral fellows, 200 technicians, 25 secretaries, 10 administrators, 21 engineering jobs, and at least 50 building operations jobs.
- Implementation of a community construction employment program, with an average of 1,260 full-time new construction jobs for each of the three years of construction
- Formation of four new start-up companies generating 50 new jobs

Completion of the new Center for Science and Medicine building is anticipated in late 2012.



http://www.mssm.edu/about-us/news-and-events/new-center-for-science-andmedicine-to-bring-health-benefits-and-job-opportunities



The Translational and Molecular Imaging Institute (TMII) Resources

The Translational and Molecular Imaging (TMII) Institute (Director, Zahi A. Fayad, PhD) will occupy $\sim 20,000$ square foot in the new Center of Science and Medicine (expected 2012) occupancy). The mission of TMII is to create, develop, validate, and apply a variety of innovative and advanced imaging technologies toward a more comprehensive understanding of and better care of the human body. TMII is responsible for coordinating and executing all in vivo imaging research at Sinai. Currently TMII has over 45 members with expertise in all aspects of translational imaging research. TMII is currently fully staffed to support all the image acquisition, image analysis, scheduling and performance of the proposed experiments. There are currently 5 faculty members and several technical and support members in the areas of cardiovascular imaging, neuroimaging, cell tracking and nanomedicine. TMII will be recruiting over the next five years 8 more faculty members to parallel the expansion of translational medicine at Mount Sinai. Mount Sinai and TMII have entered into a collaboration agreement with Siemens Medical Systems to support its effort in translational research. The new Institute will manage a variety of Siemens systems including: a whole-body 7T actively shielded MR scanner, a fully integrated simultaneous MR(3T)/PET mMR system, a 3T MR Skyra scanner and a novel CT scanner based on a clinical dual-source CT system concept with one counting detector and one conventional CT detector. In addition, TMII will have access to the following Siemens systems that will be managed by the Department of Radiology: 1.5T MR Aera scanner, PET/CT(64) Biograph mCT, and multidetector CT Somatom Definition Flash. These systems will be available for human and large animal research. Small animal imaging research will be performed on a variety of scanners: 9.4T (89mm bore size) Bruker vertical bore system, 7.0T Bruker (154mm bore size) horizontal bore scanner, a micro-SPET/CT Triumph GE Gamma Medica, a micro-PET/CT Triumph GE Gamma Medica, a CT with photon counting capabilities from Philips, a biophotonic IVIS spectrum, near-IR imager from John Frangioni, micro ultrasound Vevo 2100 from VisualSonics. All the imaging systems will be equipped with a variety of peripherals for physiological monitoring, physiological gating, for fmri experiments, drug infusion and anesthesia delivery. TMII will also have the appropriate support infrastructure for clinical and animal research including patient exam and testing rooms, hot and cold injection rooms, animal procedure rooms, radionuclide dose rooms, radionuclide laboratory, electronics laboratories, and animal holding rooms. One of the main functions of TMII is to provide the infrastructure for access to research imaging. A comprehensive set of Image modalities are supported for both human as well as animal work. Scheduling support for access to the different scanners consist of web-based online calendars as well as life telephone scheduling support. TMII also provides a central hub for image distribution and archival. At the onset we will have 32TB of online storage where all imaging data is pushed to and distributed from. The capacity will be expanded annually as needed. TMII will provide image analysis support through the Image Analysis Core. This core consists of IT personnel, software engineers, imaging physicists, research assistants and other support personnel. Expert consultation for research projects including protocol design, specialized pulse sequences, special image acquisition hardware (coils), custom made functional MRI stimulus hardware are all supported. Comprehensive project based image analysis support is also provided. Modalities supported include PET, MRI, fMRI, DTI and its variants, resting state fMRI. Image analysis training is also supported for those researchers who want to learn more about image analysis in general. Training range from regular classroom based graduate course taught by TMII faculty to hands on training on the use of specific software packages such as FSL, SPM, Brainvoyager and TMII's own in house developed software



packages. The data center has a dedicated server room which houses a larger Mac Server Cluster with 2 x 16TB of initial online storage with direct connectivity to all the imaging modalities in CSM. In addition there is also an image analysis room equipped with large viewing display and more than 15 high performance workstations open for the researcher to learn or perform image analysis. Finally, a nanomedicine laboratory will be established for the design, synthesis and evaluation of novel imaging probes and drug delivery systems.



Cardiovascular Imaging Program



The Cardiovascular Imaging Program is focused on developing and using noninvasive imaging methods that allow the early detection, prevention, and treatment of cardiovascular disease. Despite considerable therapeutic advances over the past 50 years, cardiovascular disease is the leading cause of death worldwide. This is mainly a result of the increasing prevalence of atherosclerosis, owing to the ageing population, the improved survival of patients with atherosclerotic cardiovascular disease and, above all, the widespread under-recognition and undertreatment of individuals with risk factors for atherosclerosis. Atherosclerosis is characterized by the thickening of the arterial wall to form an atherosclerotic plaque, a process in which cholesterol deposition, inflammation, extracellular-matrix formation and thrombosis have important roles (see Sanz and Fayad Nature 2008; 45:953-957). Symptoms occur late in the course of disease and are usually caused by the narrowing of the lumen of the artery, which can happen gradually (as a result of progressive plaque growth) or suddenly (as a result of plaque rupture and, subsequently, thrombosis). The resultant decrease in blood supply can affect almost any organ, although coronary heart disease and stroke are the most common consequences.

Traditionally, diagnosis of atherosclerosis was possible only at advanced stages of disease, either by directly revealing the narrowing of the arterial lumen (stenosis) or by evaluating the effect of arterial stenosis on organ perfusion. We are developing and using, new imaging approaches that allow the assessment not only of the morphology of blood vessels but also of the composition of the vessel walls, enabling atherosclerosis-associated abnormalities in the arteries (including the coronary arteries) to be observed, down to the cellular and molecular level in some cases. Some of these approaches are now in clinical use or are being tested in clinical trials, whereas others are better suited to basic (preclinical) and translational research.

Zahi A. Fayad, Ph.D.

fayed Li

Director Translational and Molecular Imaging Institute Professor of Radiology Professor of Medicine (Cardiology)





Neuro Imaging Program



The mission of the Neuro Imaging Program is to research, develop and apply new technologies to non-invasively diagnose brain diseases and to further our general understanding of normal as well as abnormal brain anatomy and function. The multidimensional Neuro Imaging Program involves studies ranging from human and non-human primates to various mouse models of human diseases. The program reflects a close collaboration between the departments of Radiology, Psychiatry, Neurology and Neuroscience.

Technological advances in imaging technologies have opened up new avenues for basic, translational and preclinical studies of brain and behavior. Our laboratories has access to the state of the art imaging modalities including 9.4T and 7.0T micro-MRI animal scanners, in-vivo animal biophotonic imaging, human 3T head and whole body 3T MRI, PET/CT as well as the latest PET/MR scanners. In addition, all scanners are equipped with peripherals necessary for functional imaging including custom made devices such as a MR compatible olfacto-meter.

The Neuro Imaging Program is actively involved with the development of novel image analysis tools such as fiber tract analysis tools for DTI/DSI data as well as algorithms for resting state functional connectivity networks.

Our research portfolio includes Schizophrenia, ADHD, Mood disorders, PTSD, TBI, Autism, Alzheimer's as well as normal brain anatomy and function with target species ranging from mice to human.

Cheuk Ying Tang, Ph.D.

Director Neurovascular Imaging Program Directory In-Vivo Molecular Imaging SRF Associate Professor of Radiology Associate Professor of Psychiatry



Nanomedicine Program



The mission of the Nanomedicine Program is to establish innovative nanotechnology based approaches for molecular imaging and targeted therapy of cancer and cardiovascular disease. To this aim we have developed a range of multifunctional nanoparticle platforms. All the platforms are based on assemblies of amphiphiles and functional materials. The main focus is on nanochemistry and novel nanoparticles as well as their application in experimental models of cardiovascular disease and cancer. Multimodal imaging is used to investigate the behavior of the new materials in vivo. The program involves close collaborations between the different research efforts within TMII.

Our nanoparticle platforms include liposomes, micelles, nanocrystal micelles, (nanocrystal) lipoproteins, lipid-coated silica, nanoemulsions and different other structures composed of amphiphiles. Some selected (out of >60) key publications are listed below:

-Gianella A et al. A multifunctional nanoemulsion platform for imaging guided therapy evaluated in experimental cancer. ACS Nano. 2011.

-Lobatto ME et al. Multimodal clinical imaging to longitudinally assess a nanomedical antiinflammatory treatment in experimental atherosclerosis. Mol Pharm. 2010.

-Mulder WJM et al. Nanoparticulate assemblies of amphiphiles and diagnostically active materials for multimodality imaging. Acc Chem Res. 2009.

-Cormode DP et al. Nanocrystal core high-density lipoproteins: a multimodality contrast agent platform. Nano Lett. 2008.

-van Schooneveld MM et al. Improved biocompatibility and pharmacokinetics of silica nanoparticles by means of a lipid coating. Nano Lett. 2008.

-Mulder WJM et al. Quantum dots with a paramagnetic coating as a bimodal molecular imaging probe. Nano Lett. 2006.

-Mulder WJM et al. MR molecular imaging and fluorescence microscopy for identification of activated tumor endothelium using a bimodal lipidic nanoparticle. FASEB J. 2005.

Willem J.M. Mulder, Ph.D.

Director Nanomedicine Laboratory Assistant Professor of Radiology





Program of Excellence in Nanotechnology (PEN)

NIH/NHLBI

TMII is extremely proud to announce the success of our application "Translational Nanomedical Therapies for Cardiac & Vascular Diseases". This application aims to establish a unique multidisciplinary Program of Excellence in Nanotechnology (PEN) by integrating the cardiovascular medicine and imaging expertise of highly productive National Heart, Lung and Blood (NHLBI) funded investigators at Mount Sinai School of Medicine, New York University, and Columbia University, with the cutting-edge biomolecular and nanomedical engineering expertise of world-renowned pioneers at Massachusetts Institute of Technology and Brigham and Women's Hospital. The overarching long-term goal of this PEN application is to establish an innovative research and training program focused on developing translational nanomedical tools for the imaging-facilitated diagnosis and minimally-invasive treatment of vascular and cardiac diseases.



Directors: Zahi A. Fayad (MSSM) and Robert S. Langer (MIT)



Cell Tracking Core



The mission of the Departmental Cell Tracking Core (DCTC) is to develop and provide novel technology to allow for non-invasive in vivo detection and tracking of transplanted cells. The program is designed to act as an active interface between the Departments of Radiology, Gene and Molecular Medicine, and Vascular Medicine. The specific aims of DCTC are as follows:

- To develop novel cell tracking probes/labels.
- To develop and validate pre-clinical diagnostic imaging methods to allow for accurate in vivo detection and longitudinal cell tracking.
- To provide consulting services to help investigators optimize cell labeling strategies.
- To perform comprehensive data analysis and aid in the interpretation of imaging results.
- To provide investigators with high quality data to allow for submission of new grant applications and/or support funded studies.

DCTC can provide the latest strategies for cell detection using Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), and Computer Tomography (CT). The recent acquisition of a clinical hybrid time-of-flight PET/MR system allows for the combination of high sensitivity PET imaging with high-spatial resolution MRI. Novel cell labels are currently being constructed that combine MRI and PET tracers to take advantage of this exciting new technology.

Over the past year DCTC has been involved in the labeling, detection and tracking of a variety of cells for several indications. For example, antigen modified monocytes, Tcells, cardiac progenitor cells, hepatocytes and stem cells have been used to monitor novel immuno- therapeutics in mice and pigs. Activated labeled dendritic cells have been used to induce anti-tumor immunity that has proven effective in the fight against HIV and Lupus. The services provided by DCTC are open to all investigators on a fee for service. Summary of current publications submitted with DCTC support:

-Adler ED et al., In vivo detection of embryonic stem cell-derived cardiovascular progenitor cells using Cy3-labeled Gadofluorine M in murine myocardium. JACC Cardiovasc Imaging. 2009 Sep;2(9):1114-22.

-Adler ED et al., The Cardiomyocyte Lineage is Critical for Optimization of Stem Cell Therapy in a Mouse Model of Myocardial Infarction. JACC Cardiovasc Imaging. 2009 Sep;2(9):1114-22.

-Briley-saeboe KC et al., Longitudinal tracking of human dendritic cells in murine models using magnetic resonance imaging. Magn Reson Med. 2010 Nov;64(5):1510-9.

-Adler ED et al., The cardiomyocyte lineage is critical for optimization of stem cell therapy in a mouse model of myocardial infarction. FASEB J. 2010 Apr;24(4):1073-81.

Karen Briley-Saebo, Ph.D.

Instructor Department of Radiology Mount Sinai School of Medicine



Image Analysis Core



Image Analysis Core: Directed by David M. Carpenter, Ph.D. Team: David M. Carpenter, Ph.D., Edmund W. Wong, M.S., Johnny Ng, M.S., Emily L. Eaves, B.A., Jessica Roman, B.A., Cheuk Y. Tang, Ph.D.

Services: Investigators that take advantage of the Image Analysis Core receive services in paradigm/experimental design, MR and peripheral protocol configuration, image analysis and visualization. Expert consultation for research projects including protocol design, specialized pulse sequences, special image acquisition hardware (coils), custom made functional MRI stimulus hardware are all supported. Comprehensive project based image analysis support is also provided. Modalities supported include PET, MRI, fMRI, DTI and its variants, and resting state fMRI. Investigators and their groups can also, if they wish, receive training on workstations in the Core that are equipped with medical imaging tools. The Core fosters a friendly and didactic environment with dedicated staff that is fully trained in these packages in order to perform analyses and assist in training collaborators.

Personnel: The Core team includes IT personnel, software engineers, imaging physicists, research assistants and other support personnel that are experts in imaging paradigm development, MR protocol configuration, and imaging analysis and presentation.

Workstations: Image Processing Workstations are equipped with state of the art image processing packages and are available for investigators and their research staff. The software packages extend from commercial/public domain imaging tools for PET, MRI, MRS, fMRI and DTI (such as SPM8, FSL, AFNI, BrainVoyager, ePrime, DTI-Studio) as well as sophisticated programs developed in-house for advanced, state-of-the-art analyses (including customized Region of Interest, DTI white matter tract tracing, fMRI functional connectivity, default/resting state network analysis).

Archival: The Core provides a central hub for image distribution and archival. A 15 Terrabyte archival system has multi-tiered backup to ensure data safety. The archival system is integrated with MRI, PET, CT and pre-clinical scanners. In addition, data is backed up from Image Processing Workstations.

Training: Image analysis training is also supported for those researchers who want to learn more about image analysis in general. Training range from regular classroom based graduate course taught by TMII faculty to hands on training on the use of specific software packages. The Core has a full training curriculum from basics of MR Physics to fMRI-DTI fusion to white matter crossing fiber modeling. The training programs have been tailored for radiologists, medical students, psychiatrists, psychologists and basic scientists.





Image Analysis Core: Techniques and Algorithms



Tang CY, Friedman JI, Carpenter DM, Novakovic V, Eaves EL, Ng J, Wu YW, Gottlieb S, Wallenstein S, Moshier E, Parrella M, White L, Bowler S, McGinn TG, Flanagan L, Davis KL, The Effects of Hypertension and Body Mass Index on Diffusion Tensor Imaging In Schizophrenia, Schizophrenia Research, 2011. In Press.

Tang CY, Carpenter DM, Eaves EL, Ng J, Ganeshalingam N, Weisel C, Lange G, and Fiedler NL, Effects of occupational solvent exposure on brain function: an fMRI study. Environmental Health Perspectives, 2011, in Press.



Carpenter D, Tang CY, Friedman JL, Hof PR, Stewart DG, Buchsbaum MS, Harvey PD, Gorman JM, Kenneth L Davis, Temporal Characteristics of Tract-Specific Anisotropy Abnormalities in Schizophrenia, Neuroreport, 2008, Sep 17;19(14):1369-72. Tang C Y, Friedman J, Shungu D, Chang L, Ernst T, Steward D, Carpenter D, Ng J, Hajianpour A, Mao X, Hof P, Buchsbaum M S, Davis K and Gorman J M, Correlations between Diffusion Tensor Imaging (DTI) and Magnetic Resonance Spectroscopy (1H MRS) in schizophrenic patients and normal controls, BMC Psychiatry 2007, 7:25 (19 Jun 2007)



Tang CY, Eaves EL, Ng JC, Carpenter DM, Mai X; Schroeder DH, Condon DH; Colom R, Haier RJ, Brain networks for working memory and factors of intelligence assessed in males and females with fMRI and DTI. Intelligence, 2010, 38: 293-303



Carpenter DM, Hof PR, Tang CY, Distinguishing Group Differences from Type I Errors in DTI Normalization, 2011 (submitted)

Tang CY, Eaves EL, Dams-O'Connor K, Ho L, Leung E, Wong E., Carpenter DM, Ng J, Gordon W, Pasinetti GM. Diffused Disconnectivity in Traumatic Brain Injury: A resting State fMRI and DTI Study. Proceedings International Society of Magnetic Resonance Imaging, 2011
Abstracts selected for oral presentation





A statin - reconstituted high-density lipoprotein nanoparticle formulation that enhances the pleiotropic anti-atherosclerotic effect of statins

Authors & Affiliations Raphaël Duivenvoorden^{1,2}, Jun Tang¹, David Izquierdo-Garcia², Wei Chen¹, Neeha Zaidi¹, Gwendalyn J. Randolph¹, David P. Cormode¹, Mark E. Lobatto^{1,2}, Erik S.G. Stroes², Valentin Fuster^{1,3}, Edward A. Fisher⁴, Zahi A. Fayad¹, Willem J.M. Mulder¹.

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Introduction In addition to serum cholesterol lowering statins have compelling pleitropic effects. However, taking advantage of these effects is hampered by low bioavailability, even at the highest dose of oral statin therapy. We addressed this issue by developing a statin – reconstituted high density lipoprotein ([S]-rHDL) nanoparticle formulation, facilitating accumulation of statin in atherosclerotic lesions. We evaluated the efficacy of [S]-rHDL infusion using longitudinal 9.4 Tesla MRI studies and extensive histology.

Methods & Results ApoE-KO mice were used, since statins are known not to alter serum lipid levels in this mouse model, which allows us to investigate the anti-inflammatory properties of statins exclusively. First, using gadolinium and fluorecent dye containing [S]-rHDL, we observed nanoparticle accumulation in atherosclerotic lesions by 9.4T-MRI, near infrared fluorescence imaging, and fluorescent microscopy, and observed uptake of nanoparticles in macrophages by flow cytometry analysis of aorta cells. Second, we investigated the efficacy of 3 months [S]-rHDL therapy. 62 ApoE KO mice were randomized to bi-weekly infusion of [S]-rHDL (15mg/kg statin, 10mg/kg ApoA1), placebo (saline infusion), orally dosed statin (15mg/kg statin daily) or bi-weekly infusion of bare rHDL nanoparticles (10mg/kg ApoA1). Longitudinally in vivo 9.4T-MRI of the abdominal aortas showed that compared to the [S]-rHDL treated group, vessel wall thickness was 16% higher in the placebo group (p=0.01), 12% higher in the oral statin group (p=0.004), and 16% higher in the rHDL group (p=0.005)at the end of the study. Histology assessment showed that atherosclerotic burden of the aortic valve area in the [S]-rHDL group was decreased by 37% compared to placebo (p=0.002), by 28% compared to rHDL (p=0.006), and there was a trend towards decrease of 17% compared to the oral statin group (p=0.06). Plague macrophage content was decreased in the [S]-rHDL group by 57% compared to placebo (p=0.001), by 37% compared to oral statin (p=0.003), and by 40% compared to rHDL (p=0.03). Third, in 43 mice we assessed the effect of short term high dose [S]-rHDL therapy. We administered four infusions within a single week of high dose [S]-rHDL (60mg/kg statin, 40mg/kg ApoA1), placebo (saline infusion), high dose rHDL (40mg/kg ApoA1), or low dose [S]-rHDL (15mg/kg statin, 10mg/kg ApoA1). Histology showed a trend towards decrease of 31% in total plaque area in the high dose [S]-rHDL group compared to placebo (p=0.053), and was decreased by 34% compared to high dose rHDL (p=0.005), and by 36% compared to the low dose [S]-rHDL group (p=0.006). Macrophage positive area in the high dose [S]-rHDL group was decreased by 84% when compared to placebo (p<0.001), by 79% compared to high dose rHDL (p=0.001), and 77% compared to low dose [S]-rHDL (p=0.002).

Conclusion By attaining statin accumulation in atherosclerotic lesions with [S]-rHDL, plaque development was halted with long term low dose therapy, and plaque macrophage content markedly reduces when short term high dose therapy is applied.

Clinical Relevance We envision that in acute coronary syndrome (ACS) patients, [S]-rHDL nanotherapy can facilitate target-specific plaque retention of high quantities of statin upon intravenous administration, and thereby can target plaque inflammation. This might be an effective strategy to bridge patients over the period of vulnerability following ACS, after which standard of care therapy, including oral statins, can sustain the decreased inflammatory level of atherosclerotic lesions.



Micro-MRI Monitoring of the normal mouse neurovasculature during aging using Micellar-based contrast agent

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- 3 Mouse Imaging Centre, Hospital for Sick Children, Toronto, Canada

Introduction

There is increasing evidence that neurovascular dysfunction may be a very important risk factor in the development of Alzheimer's Disease, an age-related condition [1, 2]. For this study, we utilized recently developed Gd-loaded micelles as a long living blood pool agent to achieve highly resolved (100um)3 isotropic angiograms in mice. This approach was used to monitor three-dimensional neurovascular changes in individual C57BL/6 wild type mice over two years of normal aging.

Methods & Results

<u>Synthesis</u>: Gd-loaded micelles were synthesized based on previously described protocol [3]. Dynamic light scattering revealed a diameter of 12-15nm per micelle thus preventing leakage from the vasculature. The micelles maintain a plasma half-life of 1.4 hrs and a relaxivity of 11.6 s-1 mM-1 at 60MHz [3] <u>MRI</u>: All in vivo studies were performed in C57BL/6 wild type mice using a 7-T Bruker micro-MRI system. All mice were anesthetized with Isoflurane and body temperature was maintained using a heating pad. <u>Data Analysis</u>: The resulting brain volumes were aligned through a series of rigid, affine, and non-linear registration steps using software provided by the Mouse Imaging Centre (Toronto, Canada) and using tools developed by the Montreal Neurological Institute (Montreal, Canada). The neurovasculature was assessed qualitatively using Maximum Intensity Projections (MIPs) and quantified through segmentation of the vasculature using a thresholding function in which the vasculature was defined as the mean signal intensity of brain tissue + 2 standard deviations.

The (100um)³ angiograms were used to visualize neurovascular details and showed a steady decrease in detectable vasculature over the 2 year of aging. This decrease was seen in the vascular volume obtained from the average brain of the both 14-16 month old mice as well as the 25 month old mice when compared to the average brain of the 2-4 month old mice (data not shown). This decrease was further visualized in 5 individual mice that were scanned at both ages. Fig 1. a&b illustrate an example of a mid-sagittal brain MRI slice obtained from the 3D MRA data set of an individual mouse followed over one year and Fig 1. c&d, the corresponding MIPs, show obvious microvascular enhancement differences in various areas of the brain (see red arrows in Fig 1. c). When using the previously defined criterion for vascular segmentation, we obtained a similar enhancement seen in the MIPs (Fig 2. a&b). The number of voxels corresponding to neurovasculature was quantified and demonstrated a significant decrease (p<0.05, two-tailed student's t-test) with age (Fig 2. c).

Conclusion

Our approach enabled effective longitudinal monitoring of neurovascular changes with excellent contrast enhancement and high anatomical detail. Quantification of the vascular volume during normal brain aging in C57BL/6 wild type mice revealed a steady and significant age-dependent decrease in the neurovasculature.

Acknowledgments: We thank Drs. Mark Henkelman, John Sled and Jason Lerch (Mouse Imaging Centre) for providing the software used for image registration and Cesar A. Berrios-Otero and Kamila Szulc from the Turnbull lab for their initial assistance with registration and image analysis. This research was supported in part by the American Health Assistance Foundation grant A2008-155 (YZW), Alzheimer Association grant IIRG-08-91618 (YZW), Tilker Medical Research Foundation (YZW), and by the NYU Applied Research Support Fund (YZW).



References: [1] de la Torre JC, Lancet Neurol 2004, 3:184 [2] ladecola C, Nat Rev Neurosci 2004, 5:347 [3] Briley-Saebo KC et al. Circulation 2008. 117(25): p3206-15 [4] Hill, LK et al, Proceedings of the 17th Annual ISMRM Meeting 2009, 3165

Clinical Relevance

This study in normal aging mice serves as a baseline for future therapeutic studies of age-dependent diseases such as Alzheimer's Disease.

Figures and tables



Fig 1- 2D mid-sagittal views from $(100 \text{ um})^3$ 3D CE-MR angiograms of an individual mouse at 4 months (a) and 16 months old (b) and corresponding MIPs (c and d, respectively) revealing a decrease in detectable vasculature after aging one year.

Fig 2: Intensity-based vascular segmentation superimposed onto a 2D mid-sagittal section at 4 months old (a) and 16 months old (b). Quantification of voxels corresponding to neurovasculature demonstrated a significant age-dependent decrease (p=0.0145); same color and shape data points represent individual mice, with the dashed lines showing the average number of vascular voxels in each age group (c).



Functional Biomaterials for MRI-based Cell Tracking

Authors & Affiliations

Erik M Shapiro

Departments of Diagnostic Radiology and Biomedical Engineering, Yale University School of Medicine

Introduction

Magnetic particles are a key enabling technology for MRI-based cell tracking. We have pioneered the use of micron sized particles of iron oxide (MPIOs) for molecular and cellular MRI. However, the inert matrices of these particles could be problematic for translation for use in humans. Furthermore, these MPIOs are in a constant 'on' state, meaning it is currently impossible to use MRI-based cell tracking of magnetically labelled cells to monitor cell processes. We have developed highly magnetic biodegradable nano- and microparticles which both have the potential for clinical translation and the ability to enzymatically change MRI properties, potentially changing from an 'off' to an 'on' state. These new particles paradigms will facilitate single cell MRI detection in humans and would be useful in monitoring the differentiation of multiple stem cell types, but also other cell types, in vivo.

Methods & Results

Polymer encapsulated magnetic particles: Magnetite nanocrystals were encapsulated in PLGA. Both nano- (100-150 nm) and microparticles (1-2 mm) were fabricated. Magnetite wt% as high as 80% was achieved and r_2^* relaxivities as high as 650 mM⁻¹s⁻¹ were measured, up to 10 times higher than commercially available materials. Cells were magnetically labelled in culture and multiple cellular assays were performed. Not only did labeled neural and mesenchymal stem cells remain viable, but they also retained their ability to differentiate into multiple lineages in culture. Furthermore, magnetically labeled immune cells were able to secrete appropriate levels of cytokines following stimulation. Lastly, these particles were proven useful in an innovative MRI-based cell tracking paradigm, *in vivo*.

<u>**RElease ACTivation of Iron Oxide Nanoparticles (REACTION)</u></u>: REACTION involves the enzymatic modulation of nanoparticle relaxivity by removing the polymer coating surrounding magnetic nanocrystals. This principle was investigated in vivo using dextran coated magnetite and dextranase. Two sets of MCF7 cells were prepared. One set was labeled only with Feridex, the other set labeled with both Feridex and dextranase as above.** Following treatment with the anti-mitotic agent mitomycin C, each cell set was injected into the hind limb muscles of CD1 nude mice. T₂* weighted MR images acquired at 1 day post injection demonstrated that a much greater magnetic susceptibility effect was generated by cells treated with dextranase and Feridex relative to Feridex only labeled cells.</u>

<u>Convertible manganese particles</u>: Manganese oxide (MnO) nanocrystals were encapsulated by PLGA to form nano- (140 nm) and microparticles (1.7 mm). Intact, these particles have very low r_1 relaxivity, < 0.5 mM⁻¹s⁻¹. Upon intracellular incorporation into low pH endo/lysosomes, the MnO dissolves to produce Mn²⁺, a strong MRI contrast agent with r_1 relaxivity of 7 mM⁻¹s⁻¹.

Conclusion

Engineered nano- and microparticles were fabricated which not only enable MRI-based cell tracking of cell transplantation, but also can potentially enable investigation of stem cell differentitaion or immune cell activation. Importantly, these particles were fabricated using largely FDA-approved materials, presenting a trajectory for FDA approval.

Clinical Relevance

Key to the promise of cellular therapies for clinical applications are methods for non-invasively detecting cell delivery, cellular migration and cell processes. As such, clinically viable, functional biomaterials are necessary.





POLYMER ENCAPSULATED MAGNETIC PARTICLES: tite:PLGA = 0:1 etite PLGA = 1:1 Particle Table 1 nanoparticles microparticles Magnetite PLGA 0:1 2.1 0.1 2:1 4. Nanoparticles Particle size, nr 105+33 05+34 105±62 2100±1100 920+510 920+790 Magnetite conten -O 56.7 83.7 0 58.2 66.1 /2 S"mM" iron 196 28.5 44.7 387 rz-s**mM** particle 8.91 x10⁴ 3.95 x10 1 937 ×10 2.11 x10¹¹ Microparticle rf* s*mM* iron 659.1 243 384.1 614.1 rs*, s'mM' particle 3.37 ×10 1.65 x10¹ 3.35 x1012 2.99 x10⁹



Top left: SEM of particles. Top right: Particle characteristics. Bottom left: MSC differentitation to adipocytes and osteocytes. Bottom right: In vivo serial MRI of rat neurogenesis with histological correlation. Magnetically labelled neuroblasts carry magnetic particles from SVZ (left) to OB (right).





dextranase). Dark contrast regions are circled in red. B) Measured volume of dark contrast regions. C) CNR from cells labeled with Feridex only or co-labeled with Feridex and dextranase

CONVERTIBLE MANGANESE PARTICLES:

feridex and red fluorescent dextranase (upper).





A) TEM of MnO cores. B) SEM of MnO MPs. C) Bright field and fluorescence microscopy overlay showing an RG2 cell labeled with green MnO MPs. D) T₁ maps of RG2 cell pellets labeled with: 1) media 2) 50% MnO NPs 3) 100% MnO NPs 4) 50% MnO MPs 5) 100% MnO MPs. E) EPR spectrum of RG2 cells incubated with 50% MnO MPs for 24 hours, showing intracellular evolution of Mn²⁺.



White Matter Correlates of Impulsivity in Patients with Mild Traumatic Brain Injury

Authors & Affiliations: Heather Berlin, PhD, MPH, David M. Carpenter, PhD, MSSM

Introduction: Patients with mild TBI (mTBI) present no observable anomalies on MRI images, yet a range of cognitive and behavioral abnormalities are repeatedly reported, including impulsivity, which has been shown to involve prefrontal cortex dysfunction. The underlying neural correlates of the cognitive and behavioral deficits reported in mTBI patients are poorly understood and since there are no macrostructural lesions in mTBI we directed our attention to the microstructure using diffusion tensor imaging (DTI). This technique is sensitive to changes in white matter microstructure that include myelination, gliosis, axonal swelling, neuronal loss as well as axonal coherence. Using DTI, we investigate the relationship between microstructural white matter damage in mTBI and impulsivity, which is commonly reported in these patients. Specifically, we hypothesized that frontal white matter integrity would correlate with impulsivity in mTBI.

Methods & Results: Seven (2 male; 5 female) participants diagnosed with mTBI and 11 (4 males; 7 females) healthy controls were scanned using DTI and administered the Barratt Impulsiveness Scale-11 (BIS-11), which measures attentional/cognitive (rapid shifts and impatience with complexity), motor (impetuous action), and non-planning (lack of future orientation) impulsivity.

All imaging was performed on an Allegra 3T head-dedicated system (Siemens, Ehrlangen, Germany). DTI was acquired with a pulsed-gradient spin-echo sequence with EPI (TR = 4100 ms, TE = 80 ms, FOV = 21 cm, matrix = 128 × 128, 28 slices, thickness = 3 mm skip 1 mm, b-factor = 1250 s/mm2, 12 gradient directions, 5 averages). DTI images were compared using two approaches: voxel-wise statistics and DTI-tractography. Voxel-wise analysis was performed using the tract-based spatial statistics (TBSS) routines implemented in FSL to compare FA between groups on the white matter skeleton image. In addition, impulsivity was tested for correlation with FA on the white matter skeleton image via TBSS. Brute-force streamline DTI tractography was implemented in Matlab and used to quantify diffusion parameters of the forceps minor.

First, the voxel-wise TBSS analysis revealed large scale, non-specific decreased FA and increased MD throughout the white matter (p < 0.01, FWE corrected for multiple comparisons; Figure 1a). The second analysis that was performed was to look at the behavioral correlates of impulsivity in the patients exposed to mTBI. There were no significant between group differences in all subtests of the BIS-11 with the exception of the non-planning scale, where mTBI subjects (M=26.43, SD=5.127) were significantly more impulsive than controls (M=21.55, SD=4.228), t(2.202)=4.883, p<.05. Thus, the BIS non-planning metric of impulsivity was entered into a voxelwise correlation analysis with white matter FA. FA in the frontal lobe white matter of the forceps minor and genu of the corpus callosum showed a trend towards an inverse correlation with impulsivity as measured by the BIS (p < 0.01; FWE corrected for multiple comparisons; Figure 1b). DTI tractography was used to quantify tract specific FA of the forceps minor (Figure 2). The forceps minor FA negatively correlated with the BIS impulsivity index (r = -0.945, p = 0.001; Figure 3).

Conclusion & Clinical Relevance: Using DTI-tractography and voxel-wise statistics we showed that FA was reduced in a population of patients with mTBI and that the degree of FA reduction in frontal lobe white matter correlated with increased non-planning impulsivity. These findings are in line with previous research that implicates prefrontal cortex dysfunction in impulsivity, and provide a potential structural explanation for some of the behavioral changes commonly reported in patients with mTBI, in particular impulsivity. Many of the chronic cognitive and behavioral deficits in individuals with mTBI may arise from the disruption of white matter connectivity. These white matter tract lesions are located predominantly in anterior cerebral areas, which are selectively vulnerable to rotational shearing injury. Our findings also suggest that cognitive-behavioral therapies aimed at enhancing prefrontal cortex/executive function may be especially effective for patients with mTBI.



Figures and tables

Figure 1a



Figure 1b



Figure 1a. FA is significantly reduced in patients with mTBI (p < 0.01, FWE corrected for multiple comparisons). Orange indicates lower FA in patients compared to controls. No areas showed the converse. Figure 1b. Voxel-wise white matter FA correlations with BIS non-planning impulsivity index. Significant correlations are shown in red/yellow (p < 0.01; FWE corrected for multiple comparisons).



Figure 2. Quantitative DTI tractography of the forceps minor.



Figure 3. Forceps Minor FA of patients with mTBI correlated inversely with the BIS non-planning impulsivity index (r = -0.945, p = 0.001, n = 7).

Abstracts, Cardiovascular Imaging category





Monitoring statin therapy in atherosclerotic rabbit using P904-enhanced MRI and

FDG-PET on a new hybrid PET/MRI system.

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Introduction: Rupture of atherosclerotic plaques may trigger the onset of clinical events like myocardial infarction or stroke. Macrophages are inflammatory cells that have been demonstrated to contribute to plaque instability/rupture. Thus, visualization of the macrophage burden could be a beneficial tool to monitor the efficacy of therapeutic interventions. Fluorodeoxyglucose positron emission tomography (FDG-PET) and contrast-enhanced magnetic resonance imaging (MRI) are two imaging techniques that have shown potential in visualizing the inflammation/macrophage burden in atherosclerotic plaques via uptake of FDG and ultra-small superparamagnetic iron oxide (USPIO) respectively. Recently, Phillips introduced a combined PET/MRI hybrid system that allows sequential in vivo acquisition of PET and MRI scans. In the current study, we aim to monitor the effects of statins, a lipid-lowering therapy proven to diminish the inflammation burden, in atherosclerotic rabbits using PET/MRI. A newly developed USPIO (P904, Guerbet, France) will be used to obtain contrast-enhanced MR images of the inflammation burden of atherosclerotic plaques while FDG-PET/MRI will be used to assess their macrophage metabolic activity.

Methods: Atherosclerotic plaques were induced in rabbits using the double ballon injury model. All animals underwent USPIO-enhanced MRI and FDG-PET/MRI at baseline and were divided in a control and a statin treated group (n=3 and n=4 respectively). Both groups were scanned again 6 months after baseline imaging. The imaging protocol included a 3T MRI of the abdominal aorta prior and 24 hours after P904 injection. We applied a high-resolution gradient echo T2*-weighted sequence with 16 echo times ranging from 4.8 ms to 38.5 ms. FDG-PET/MRI was performed immediately before the 24h post-P904 MRI. T2* maps were generated in the aortic wall and R2* values (R2*=1/T2*) were calculated pre/post P904 injection and averaged over the entire aorta. R2* post-P904 injection were compared at baseline and 6 months (delta R2*) in both groups to monitor an eventual decrease in USPIO uptake after treatment. FDG-PET/MRI data were analyzed by averaging the mean SUV values over the entire abdominal aorta and compared between both groups. The mean SUV values were correlated with the macrophage density in the plaques detected by immunohistochemical staining (RAM-11) while USPIOs were detected with PERL iron staining. Finally, to study the correlation between the results obtained with USPIO-enhanced MRI and FDG-PET/MRI, we plotted R2* against the mean SUV measured in each animal.

Results: At baseline, P904 injection induced a strong darkening of the vessel wall area compared to pre-injection imaging. A shortening in T2* relaxation times due to the presence of iron in the vessel wall was observed (Figure 1A, B). This was confirmed by an increase of the R2* average values in the aortic wall from 0.021 s^{-1} pre-P904 to 0.042 s^{-1} post P904 in the progression group and from 0.025 s^{-1} to 0.054 s^{-1} in the regression group. FDG-PET/MRI at baseline revealed a strong uptake of FDG in the abdominal aorta (Figure 1C). At 6 months, mean SUV values measured in the statin group showed a significantly reduced uptake of FDG with a mean SUV decreased by 36% compared to baseline. Although not significant, R2* values calculated after P904 at 6 months showed a similar decreasing suggesting less iron uptake in the aortic wall of statin treated animals. These finding reflect a decrease of macrophage metabolic activity and density in the plaques. The latter was quantified by RAM-11 immunohistochemistry (Figure 2C, D, F) and showed a good correlation with the mean SUV (Figure 2H). In addition, USPIOs detected by PERL staining proved to colocalize with macrophages in the aortic wall (Figure 3). Interestingly, the R2* values measured after injection of P904 correlated well with the FDG uptake on PET/MRI (R²=0.5877) (Figure 4) suggesting that the intensity of phagocytosis and metabolic activity are closely associated in activated macrophages.

Conclusion: This study is the first to demonstrate the use of a combined PET/MRI system to monitor the effects of statin therapy in atherosclerotic rabbits. We have demonstrated that FDG-PET and USPIO-enhanced MRI allowed the (semi)-quantitative evaluation of plaque inflammation.

Clinical Relevance: Inflammation has been demonstrated to play a critical role in the progression of atherosclerotic plaques. This study suggests a role for combined FDG-PET/MRI in the identification of high-risk patients susceptible to suffer from plaque rupture and in the monitoring of atherosclerosis treatment efficacy.



MOUNT SINAI SCHOOL OF MEDICINE INSTITUTE T2* map Pre-P904 T2* map Post-P904





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ies 302 / S

D

F Mac

Н

PERL iron staining



Fused FDG-PET/MRI - Progression 6 Months



RAM-11 macrophages - progression 6 months



E Mean SUV in prog rabbits versus statins treated rabbits







RAM-11 macrophages - regression 6 months

Post P904 MRI



RAM.11



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Figure 1

Fused FDG-PET/MRI - Regression 6 Months



p=0.0032

abbits versus statin treated rabbits

Figure 2

Figure 3

Figure 4



In Vivo MRA quantification of vessel deformation of infrapopliteal arteries in rest and flexion.

Authors & Affiliations

Adam Oberlander, Daniel Samber, Stuart Cohen, Robert Lookstein, Joshua Weintraub, Scott Nowakowski, Edward Kim, Aaron Fischman

Introduction

The goal of this study was to quantify in vivo deformations of the infrapopliteal arteries during maximum knee and hip flexion using magnetic resonance (MR) angiography to improve the description of the complex, dynamic, below the knee (BTK) environment.

Methods

Contrast enhanced MR angiography was performed of the infrapopliteal arterial circulation in thirteen healthy adults at rest in the supine position and with knee and ankle plantar flexion. The infrapoplieal arteries were defined as the centerline path from the origin of the anterior tibial (AT) artery to the dorsalis pedis artery and the tibioperoneal trunk to the posterior tibial (PT) artery below the ankle. Deformations that resulted from the supine position to the flexed position were quantified for the three infrapopliteal arterial paths. Vessel lengths were calculated at rest and flexion for the AT and PT vessels. Area between the AT and PT, AT and peroneal, and PT and peroneal were calculated. Radius of curvature for the AT and PT at the ankle were calculated at rest and flexion.

Results

Twenty six limbs were evaluated. Area between the anterior tibial and posterior tibial arteries changed from rest to flexion an average of 10% +/-10.2%. Area between the AT and peroneal changed an average of 18.1% +/- 13.6%. Area between the PT and peroneal changed an average of 10.7% +/-9.2%. Vessel length changes were observed from rest to flexion for the AT and PT. The AT length changed an average of 19mm (5.1%). The PT length changed an average of 2.4mm (0.4%). The radius of curvature changes were recorded for 19 arteries at the level of the ankle at rest and flexion. The distal AT and proximal dorsalis pedis changed an average of 151% +/-34%. The distal PT at the ankle changed an average of 25% +/- 24%.

Conclusion

The data show that leg flexion causes infrapopliteal arteries to change orientation substantially including widening of the area between the AT and PT with significant length and angulation changes. These findings suggest significant dynamic forces in the infrapopliteal arteries and provide important parameters for stent design and possible fracture mechanisms.

Clinical Relevance

Although the benefits of implanting intraluminal stents into diseased blood vessels are immediate and obvious, the long-term effects of the procedure are subject to a number of potential complications. The phenomenon of stent fracture and (and subsequent in-stent restenosis) is a somewhat rare but serious complication with a poorly understood etiology. One potential cause may result from the deformations of the vessel in question during routine movement. The purpose of this study was to measure and characterize vessel biomechanics in order to better understand and evaluate this association.







Length analysis was performed from the origin of the vessel to the level of the ankle

Results: Length of Vessel				
	Rest Mean +/- STD	Flexion Mean +/- STD	Mean ∆ length	%Δ
Left AT	352mm +/- 28	369mm +/- 28	17 mm	4.7%
Peroneal	307mm +/- 35	314mm +/- 52	7 mm	2.2%
PT	346mm +/- 50	352mm +/- 49	6 mm	2.0 %
Right AT	369mm +/- 34	367mm +/- 36	- 2 mm	- 0.1%
Peroneal	288mm +/- 33	283mm +/- 67	- 10 mm	- 2.8%
PT	356mm +/- 29	366mm +/- 28	10 mm	3.0%



	Rest Mean Δ	Flex Mean	Change	%Δ
Section 20	+/- STD	+/- STD	+/- STD	+/- STD
Left AT-Per	2621mm ² +/- 589	2107mm ² +/- 463	514mm ² +/- 505	18% +/-17%
Pt-Per	6709mm ² +/- 1,469	6260mm ² +/- 1,629mm	449mm ² +/- 817	6% +/-13%
АТ-РТ	14289mm ² +/- 2661	12609mm ² +/- 2208	1681mm ² +/- 1354	11% +/- 8%
Right AT-Per	2482mm ² +/- 727	2,020mm +/- 398	514mm +/- 505	18% +/-17%
PT-Per	6197mm +/- 1211	5,718mm +/- 1191	478 +/- 805	7% +/-13%
At-PT	14,141mm +/- 2545	12714mm +/- 1787	1427mm +/- 1787	8% +/-11%

Radius of Curvature

e more sharply curved the vessel is, the smaller the radius of curvature.





Rest Right PT 97°



Results: Radius of Curvature (ROC)				
ROC -PT- A	ROC -AT- A	ROC -Peroneal- Δ		
25%	150%	38%		
<u>n</u> = 16	<u>n</u> =2	<u>n</u> =1		



The relationship between dynamic contrast enhanced (DCE) magnetic resonance imaging (MRI) and 18F-FDG positron emission tomography (PET) in subjects with carotid atherosclerosis

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Introduction Plaque inflammation is characterized histologically by the presence of active macrophages and neovessels, both hallmarks of vulnerable plaques. ¹⁸F-FDG PET/CT (*Tawakol et al, JACC, 2006*) and DCE-MRI (*Kerwin et al, Circulation,2003; Calcagno et al, ATVB, 2008*) are two non-invasive imaging techniques that report on, respectively, plaque macrophages and neovessels, a certain degree of correlation between ¹⁸F-FDG PET/CT and DCE-MRI outcomes can be expected. If true, this notion would impact their mutual role in assessing plaque inflammation in future clinical practice. Here we investigate the relationship between DCE-MRI and ¹⁸F-FDG PET/CT in 23 patients with carotid atherosclerosis.

Methods & Results: Methods: 23 subjects with documented vascular disease were recruited across four imaging sites. DCE-MRI was performed on 1.5T whole body systems on one axial slice using a black blood TSE sequence. After motion correction and de-noising, signal intensity curves were converted to concentration. A modified Tofts model (Figure 1) and non-modelling based approaches (area under the curve, AUC) were used to assess the uptake of contrast agent in the vessel wall. After 120 minutes of FDG circulation time, carotid PET imaging was acquired in 3D mode, covering one bed position. PET/CT data were analyzed by calculating the slice by slice standardized uptake value (SUV) and target to background ratio (TBR, SUV corrected by the blood pool activity). After automated registration, DCE-MRI and PET/CT outcome variables from corresponding slices were correlated using Pearson's correlation. Right and left carotid arteries from the same patient were treated as independent. Results: All 23 subjects had analyzable PET/CT images, thus yielding 46 analyzable carotids. As for DCE-MRI, data from five vessels out of 46 were not analyzable. Out of the analyzable vessels, 10 data points were excluded due to fitting failure during kinetic modeling. Therefore a total of 31 vessels were compared to PET/CT. A significant negative correlation was found between the DCE-MRI kinetic parameter K_{ep} and the average carotid TBR (R=-0.316, p=0.044) (Figure 2). K_{ep} representes the constant describing the exchange of contrast agent from the tissue compartment back to the plasma compartment and is defined as K^{trans} (the constant describing the exchange of contrast agent from the plasma to the tissue compartment) divided by v_e (the fraction of extravascular extracellular space). No significant correlation was found between other DCE-MRI outcomes (AUC, K^{trans} , v_e and v_p) and either mean or maximum SUV or TBR.

Conclusion In this study, we show a significant negative correlation between a DCE-MRI outcome (K_{ep}) and average vessel TBR as quantified by PET/CT. This finding is in agreement with studies performed in tumors (*van Laarhoven et al, Radiology, 2005*). There, a positive correlation was found between tumors neovessels density and K_{ep} , while a negative correlation was exploited between K_{ep} and average tumor TBR. This was taken as an indication that higher uptake of FDG would indicated hypoxic areas in the tissue. This hypothesis could be also valid in the case of ahterosclerotic plaques, although confirmation by histology will be needed.

Clinical Relevance The presence of active macrophages and neovascularization are hallmarks of of vulnerable atherosclerotic plaques and can be characterized non-invasively with ¹⁸F-FDG PET/CT and DCE-MRI. Based on the tight relationship between the two histological variables, in this preliminary study we explore the correlation between ¹⁸F-FDG PET/CT and DCE-MRI. This knowledge will impact the mutual role of these techniques in future clinical practice to assess atherosclerotic plaques.



Figures and tables



Figure 1: A) arterial input function (red line) and tissue curve of one representative carotid artery (blue line). B) tissue curve of one representative carotid artery (blue line) and corresponding fitting (dashed red line). X axis, time (min). Y axis, concentration (mmol)



Figure 2: Correlation between DCE-MRI and PET/CT. X axis, Kep (1/min). Y axis, TBR mean. Blue dots represent indiviaul data points. Black dashed line, fitting line.



Manganese G8 Dendrimers for the in-vivo Detection of Oxidative Epitopes in Atherosclerotic Plaques

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Introduction: Oxidized lipoproteins (OxLDL) play key roles in a number of inflammatory pathways that contribute to atherogenesis, including the recruitment and activation of monocytes into macrophages. Postmortem studies indicate that high levels of OxLDL epitopes, namely malondialdehyde (MDA)-lysine, are prevalent in the culprit lesions of humans, where they localize inside macrophage "foam" cells. Previously, we have used gadolinium (Gd) based micelles as a platform for the in-vivo detection of MDA-lysine epitopes using Magnetic Resonance Imaging (MRI), but the bio-retention and biotransformation of Gd may limit its clinical translatability. Manganese (Mn(II)) is an endogenous paramagnetic metal ion that is FDA approved for intracellular uptake. However, its effectiveness is dependent on its interaction with metalloproteinases and/or cell-membranes and particularly the size of the payload delivered. We therefore synthesized and characterized a biocompatiple Mn based probe using Poly(amido amine) (PAMAM) dendrimers to deliver large amounts of Mn while maintaining small particle size (<25 nm) to allow for luminal diffusion and macrophage uptake.

Methods & Results:

<u>Synthesis</u> (Figure 1): Untargeted 8th generation (G8) PAMAM dendrimers were modified to contain approximately one thousand manganese (II) ions (as chelated MnDTPA) per dendrimer. The remaining 42 amine groups associated with the G8 dendrimer were used to covalently link the dendrimer to murine monoclonal antibodies for MDA-lysine (MDA) via interaction between thiol and maleimide groups.

<u>Characterization</u>: Particles were characterized with respect to particle size, longitudinal and transverse relaxivites, in-vitro macrophage uptake and cell viability. **Table 1** shows the hydrated particle diameters and relaxivities associated with the untargeted and MDA2 dendrimers. The diameter of targeted MDA2 dendrimers (13.34 \pm 1.2 nm) is similar to that of endogenous lipoproteins, as well as gadolinium micelles that were previously developed. No significant difference in the relaxation were observed between the untargeted and targeted dendrimers in buffer and wild-type mice blood, but significant differences were observed in apoE-/-mouse blood, as expected. ICP-MS results showed limited uptake of untargeted dendrimers and significant uptake of targeted MDA-targeted demdrimers in J744A.1 macrophages for concentrations ranging from .08 to 1 pg Mn/cell. Incubation of J744A.1 macrophages with targeted and untarged dendrimers showed no significant variation in viable cell proportions after incubation with targeted dendrimers (77.8%), targeted dendrimers (74.3%), or control saline (62.6%).

<u>In-vivo Imaging</u>: ApoE-/- mice received a pre-injection MRI scan prior to tail vein tail vein administration of a .05 mmol Mn/kg dose of either targeted or untargeted MDA2-dendrimers. Each mouse was subsequently underwent post-injection scans at 24HRS, 48HRS, and 72HRS. MR imaging of the abdominal aorta was performed using a T1-weighted black blood spin echo sequence (TR/TE = 667 ms/9.9 ms, averages =10, FOV = 2.5x2.5 cm, slice thickness =.5 mm, number of slices = 30) on a clinical 3 Tesla hybrid time-of-flight PET/MR system. The targeted dendrimers exhibited significantly greater arterial wall enhancement relative to the untargeted material at all points post-injection (Figure 2). Histology on matched aorta sections displayed strong correlation between oxLDL deposition and MR signal enhancement.

Conclusion: Our study demonstrates the feasibility of using manganese labelled dendrimers targeted to oxidation-specific epitopes for in the vivo imaging of vulnerable atherosclerotic lesions.

Clinical Relevance: In the United States, 68% of the 800,000 annual CVD-related deaths are attributed to coronary heart disease and stroke, complications of atherosclerotic lesions. New aggressive therapies and interventions have recently emerged that promise to promote lesion regression and/or prevent the structural compromises of high risk atherosclerotic lesions. In order to evaluate the efficacy of these emerging therapies, non-invasive diagnostic imaging techniques that surpass the morphologic and anatomical evaluation are required. The use of molecular imaging probes can enable cellular and molecular events in atherosclerotic lesions to be detected and quantified.



Table 1 : Physical and chemical properties of the dendrimer formulations. WT = wild type mouse plasma.					
APOE-/- = apolipoprotein deficient mouse plasma. All longitudinal relaxivities, r1, determined at 60 MHz					
and 40 ° C.					
Formulation	Size + sd	r1 (s ⁻¹ mM ⁻¹)	r1 (s ⁻¹ mM ⁻¹)	r1 (s ⁻¹ mM ⁻¹)	

Formulation	Size ± sd	r1 (s mM)	r1 (s mM)	r1 (s mM)
	(nm)	Buffer	WT plasma	ApoE-/- plasma
Untargeted	11.6 ± 0.1	3.4 ± 0.1	3.8 ± 0.1	4.0 ± 0.3
MDA2	13.3 ± 1.2	3.5 ± 0.1	4.0 ± 0.2	5.5 ± 0.4





Role of hypoxia on neovascularization and inflammation of the atherosclerotic plaque

Authors & Affiliations

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Introduction

Recent evidence has associated plaque angiogenesis with enhanced atherosclerosis progression and vulnerability. It is well established that hypoxia is the main stimulus for angiogenesis, both physiologically and pathologically. We hypothesize that the neovascularization of the atherosclerotic plaque is mainly driven by the hypoxic component of the plaque, primarily due to HIF-1 α expression (Hypoxia Inducible Factor-1 α). The aim of this project is to investigate the role of hypoxia during the development of atherosclerosis and explore the contribution of the inflammatory cells to the generation of hypoxia inside the plaque in a rabbit model of aortic atherosclerosis, using non-invasive imaging modalities (PET and MRI) and immune-detection techniques.

Methods & Results

Advanced aortic atherosclerotic lesions were induced in New Zealand White rabbits (n=18) by a combination of atherogenic diet (0.2% cholesterol) and a double aortic endothelial balloon denudation. To evaluate the influence of tissue hypoxia on atherosclerosis development and progression, the animals undergo sequential PET/MR imaging after the administration of ¹⁸F-Fluoromisonidazole (¹⁸F-FMISO), which allows the quantification of tissue hypoxia ($[O_2] \le 1\%$). Fortyeight to seventy-two hours later, the animals are subjected to ¹⁸F-Fluorodeoxy-glucose (¹⁸F-FDG)-PET/MR imaging to estimate plaque inflammation, followed by Dynamic Contrast Enhanced MRI (DCE-MRI) to detect plaque composition and neovessels content by the injection of the MRI-contrast agent Gadolinium (Gd-DTPA). At each study time-point (8 and 12 months after initiating the diet) a group of animals are euthanized 2-hours after the injection of pimonidazole (hypoxia marker), and aortas processed for histopathological evaluation of plaque hypoxia (pimonidazole), macrophages content (RAM-11), neovascularization (isolectin B), and HIF-1 α expression. After 8-months on diet, we observe an increase of ¹⁸F-FDG uptake in some portions of the aorta that correlates with strong staining of RAM-11. Also, we have detected positive staining for pimonidazole in some areas of the aorta as well as a trend of increase of ¹⁸F-FMISO uptake, in comparison with healthy control rabbits. Remarkably, with the current data (8 months) we do not observe a clear correlation between macrophages and hypoxia staining. Additionally, the DCE-MRI experiments show an enhancement of the arterial wall signal intensity in cholesterol-fed animals, which will allow us to quantify plaque neovascularization.

Conclusion

At early stages of the disease (8 months) no clear correspondence between hypoxia and macrophages content is observed. A second time-point (12 months) is expected to give us a better understanding of the correlations among hypoxia, inflammation and neovascularization of the atherosclerotic plaque.

Clinical Relevance

¹⁸F-FMISO-PET may allow for the evaluation of hypoxia in atherosclerotic plaques. This non-invasive imaging modality could be proposed as a clinical tool in the evaluation of lesion prognosis and monitoring of anti-angiogenic therapies.



Ex vivo imaging of cardiovascular fibrosis with biomarker fluorescent probes

Authors & Affiliations

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Introduction

Fibrosis plays an important role in hypertrophy and congestive heart failure. The collagen-binding adhesion protein CNA35 is appearing as a promising small molecular agent for optical imaging of collagen in vitro and in vivo. The present study aims to characterize CNA35 on cardiovascular fibrosis in hypertrophy and ischemic coronary atherosclerosis in rat.

Methods & Results

Thirty two Sprague-Dawley male rats were used in the study. Animals underwent ascending aortic banding four months to induce hypertrophy. Neointima in coronary arteries was induced by aortic banding for two months following coronary arterial ischemia/reperfusion one month and de-aortic banding one month. Cardiovascular fibrosis in frozen OCT tissue section was detected with recombinant CNA35 probe which was labeled with cyanine dye (Cy3). A biphenylalanine myocardial targeting peptide (MTP) conjugated with FITC was used to counterstain myocardium. The CNA35 fluorescent imagings on interstitial and perivascular fibrosis, neointima and infarct scar were compared with traditional Masson's Trichrome and Picrosirius Red stain. Fluorescent CNA35 probe shows its advantages in assessment of fibrosis vs traditional stains: easy to perform, fast, multi-chromatic and much less toxic. Interstitial and perivascular fibrosis were increased 5-6 folds in hypertrophic (n=8) and ischemic de-overload hearts (n=8) compared with control animal (n=6). Fifty percent of ischemic de-overload hearts (4/8) had neointima in coronary arteries. Bundle collagen fibers could be identified through CNA35/BMTP probes in isolated myocytes from adult rat in vitro.

Conclusion

CNA35 is a new promising fluorescent probe in assessment of cardiovascular fibrosis in vitro.



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Development of a Rabbit Model of Lipid-Rich Soft Plaque and Non-Invasive Vascular Imaging with FDG PET / MR Angiography: Atherosclerosis Development Following Acyl-Conezyme A: Cholesterol Acyltransferase (ACAT) Inhibitor Treatment with DUP-128

Authors & Affiliations

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Introduction

Evaluation of the lipid-rich soft plaque in atherosclerotic lesions is clinically important for stratifying risk and providing early treatment. Non-invasive imaging with positron emission tomography (PET) is able to detect atherosclerotic lesions along with bio-pathologic functions. The goals of this study are 1) to evaluate drug efficacy of ACAT inhibitor (DUP-128) using non-invasive imaging of atherosclerotic plaque in the injured rabbit model with co-registration images of FDG PET / magnetic resonance angiography (MRA), and 2) to confirm a pharmacodynamic imaging biomarker in the atherosclerotic rabbit model for future drug efficacy studies.

Methods & Results

In thirty New Zealand white rabbits (male, 3.4 ± 0.2 kg), an endothelial balloon denudation procedure was performed using four repeat pullbacks to enhance the plaque formation following one week feed on high fat/high cholesterol (HF/HC) diet. At the end of 8 weeks of HF/HC feeding, six rabbits were euthanized for lesion characterization prior to treatment. The other twenty four rabbits were re-grouped to control-diet-only (n=12) or ACAT inhibitor treatment (30 mg/kg, n=12). The ACAT inhibitor was administered via diet admixture. Six rabbits from each group were selected for FDG PET/MRA imaging longitudinally at Baseline, Interim (4 weeks), and Final (8 weeks) time-points. The remaining six rabbits were used for histological analysis at the 4 week time point. After final imaging was acquired, all remaining animals were sacrificed and processed for histology. In addition, macrophage density was measured by immunohistology (RAM 11 and ABCA1) on corresponding aortic cross-sections.

Axial 2D MRA was performed on Bruker Biospec 47/40 MRI system, using the FL2D sequence, for visualization of blood vessels in the area of interest. FDG PET images were acquired using a dedicated primate microPET F220 scanner. At 130 minutes after FDG ($3.5 \pm 0.4 \text{ mCi}$) injection via a marginal ear vein, a 30 minute transmission scan was performed for the entire surgical area of rabbit followed by a 30 minute emission scan. Each PET image was co-registered with its corresponding MRA image guided by three fiducial (~ 2 μ Ci of FDG in 0.2% Gd-DTPA each) markers and anatomical landmarks. The region of interest (ROI) encompassing the abdominal aorta was manually drawn in axial MRA images and then transferred to FDG PET images. Mean standardized uptake value (SUV) and target-to-blood ratio (TBR = SUV in aorta / SUV in plasma) were measured on PET transverse slices.

The mean TBR ratios between Interim vs. Baseline were 0.92 ± 0.23 for treatment group and 1.33 ± 0.62 for control group. Additionally, mean TBR ratios between Final vs. Baseline were 0.75 ± 0.23 for treatment group and 1.15 ± 0.41 for control (p = 0.06). The reductions in macrophage's density in RAM 11 and ABCA1 were also observed at the Final (8 weeks) time point.

Conclusion

The aortic balloon denudation model and imaging biomarker was developed in-house for atherosclerosis evaluation. The efficacy of ACAT inhibitor (DUP-128) was successful for the



confirmation of decreasing macrophages in the abdominal aorta. The density of macrophages in the abdominal aorta of injured rabbit positively correlated with metabolic activity evaluated using FDG PET. Therefore, aortic balloon denudation and FDG PET/MR angiography can be used for the future efficacy studies.

Clinical Relevance

FDG PET/MR angiography techniques can be used to assess the total plaque burden of patients and to monitor medical intervention.

Figures and tables





Macrophage detection in atherosclerosis using targeted gold nanoparticles and computed tomography

Authors & Affiliations

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Introduction

Atherosclerosis is characterized by the accumulation of low-density lipoprotein (LDL) and recruited macrophages within arterial walls. High macrophage burden is an indicator of greater risk of atherosclerotic tissue rupture and heart attack. As computed tomography (CT) imaging is the best technique for imaging plaque in the coronary arteries, a CT contrast agent able to detect macrophages in the arteries could help identify patients at higher risk. This study investigated whether in vivo macrophage imaging using clinical scanners could be performed with gold core high-density lipoprotein (Au-HDL), a macrophage targeted agent (A). Additionally, lowest effective dose and ideal imaging timeframe of Au-HDL was probed.

Methods & Results

Dodecanethiol coated gold cores were prepared following Brust's method and subsequently coated with the phospholipid 1-myristoyl-2-hydroxy-sn-glycero- phosphocholine (MHPC). The nanoparticles were then purified through centrifugation to remove gold core aggregates and empty MHPC micelles. Negative stain transmission electron microscopy (TEM) images verified removal of empty MHPC micelles from solution after ultracentrifugation (B). Apolipoprotein A-I (ApoA-I) was added to form the final Au-HDL nanoparticle. CT imaging was used to calculate gold concentrations of the samples in mg/ml. To induce atherosclerosis, male New Zealand white rabbits were fed a high fat, high cholesterol diet (4.7% coconut oil and 0.3% cholesterol enriched diet) and underwent a double balloon injury of the aorta. Au-HDL was prepared such that five atherosclerotic rabbits were injected with 75 (n=2), 150 (n=2), or 300 (n=1) mg Au/kg. CT images of rabbit aortas were taken at the following three time points: pre-injection, 24 hours post-injection, and 48 hours post-injection. A custom made MATLAB program was created to measure various regions of and around the aorta. CT images of aorta walls after injection exhibited greater radiodensity compared to pre-injection images. For example, CT images taken of one rabbit injected with 150 mg Au/kg showed the radiodensity of the aorta on average to be 38 HU (Hounsfield Units) ± 1.99 pre-injection and 59 HU ± 1.59 24 hours post-injection (C). The lowest effective dose tested was 75 mg Au/kg and best imaging timeframe tested was 24 hours post-injection. TEM images of rabbit aorta sections confirmed localization of Au-HDL nanoparticles in macrophages (D).

Conclusion

Au-HDL increased radiodensity in CT images of aortas 24 and 48 hours post-injection compared to pre-injection images. Electron microscopy showed the nanoparticles to target macrophages. Hence this agent can image macrophages using CT, and has the potential for doing so in patients.

Clinical Relevance

If translated clinically, Au-HDL can be used to image plaques in human aortas with high macrophage burden, thus allowing identification of patients at high risk of a heart attack. In addition, the agent could be of use in studying atherosclerosis and the effect of interventions.



Figures and tables

A) Schematic of Au-HDL nanoparticle. B) Negative stain TEM images were taken of gold core nanoparticles before (1) and after (2) ultracentrifuge spin. C) CT images of rabbit aorta (circled in red) before (1) and 24 hours after being injected with 150 mg Au/kg (2). D) TEM images of Au-HDL localized within macrophages in rabbit aorta sections.





MR-derived Attenuation Maps for Whole-body PET/MR Attenuation Correction: Preliminary Results

Authors & Affiliations

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Introduction

Positron emission tomography (PET), with the ability to detect picomolar concentrations of radiolabels and magnetic resonance imaging (MRI), which provides detailed anatomic images but is limited to detecting relatively high probe concentrations allows researchers to use both conventional *in vivo* imaging techniques to examine biological processes. The use of PET/MR scanners instead of PET/CT scanners reduces the extra radiation dose to the patient but also offers higher soft tissue contrast, allowing better visualization and understanding of the underlying disease. In particular, combined Whole-Body PET/MR may be a very valuable tool for cardiovascular disease imaging, enhancing the detectability and the diagnostic of the damaged vascular beds. PET/CT attenuation correction (AC) is relatively straightforward because CT scans can be easily transferred to an attenuation map at 511 kVp for use in PET reconstruction algorithms. MRI, in comparison, has no direct information about photon attenuation but rather measures proton densities and magnetic relaxation times. The coregistration of MR data to CT data allows derivation of MR-derived AC maps. PET reconstruction comparing MRAC and CTAC dervied attenuation maps were analyzed for registration errors and intensity differences.

Methods & Results

Segmentation of human MR data into three tissue classifications, lung, soft tissue and air, and two classifications for preclinical rabbits, contour and air, was compared with the gold standard CT attenuation maps. It is clear visually that the MR attenuation map (Figure 1b) misrepresents underlying tissue structures. The percent error (Figure 1c) was calculated from comparison of the MR attenuation map to the CT attenuation map (Figure 1a). The percent difference image clearly demonstrates areas of error in regions where non-soft tissue regions that are estimated as soft-tissue show the highest regions of error. In order to quantify how these segmentation errors in the MR attenuation map may affect PET reconstruction, difference projection images were calculated (Figure 2). As expected, errors in the reconstructed PET images are situated in the region where the errors in the attenuation map are located. In parts of the body that are estimated as soft tissue, and definitely in the lungs, large overestimation in image reconstruction is very clear.

Conclusion

Despite the benefits of PET/MR in comparison to PET/CT, it is clear that improvements in MR sequence design to more accurately provide MR data for segmentation algorithms is needed. This work is currently in progress and will allow for more accurate quantification of PET tracer uptake.

Clinical Relevance

In order to reconstruct PET images, the spatial distribution of attenuation must be measured in order to permit quantitative evaluation of standard uptake values (SUV) in reconstructed images. Accurate attenuation correction using MR data must be improved in order to accurately quantify PET tracer uptake in PET/MR before such whole-body systems are adapted for clinical use.



Figures and tables



Figure 1 CT attenuation map (a), MR attenuation map classified as lung and soft tissue (b), percent difference of MR vs CT attenuation maps (c)



Collaboration with Ghent University

Figure 2 Difference projection images depicting percent errors when asssigning tissue attenuation properties in MR attenuation maps with alternate tissue attenuation values.



Assessment of Imaging Strategies for Measurement of Arterial Input Functions in Dual Imaging Dynamic Contrast Enhancement (DCE) Magnetic Resonance Imaging (MRI) Applications in the Human Carotid Arteries

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Introduction Recent developments in the study of atherosclerosis have identified the importance of inflammation, both the presence of inflammatory cells and neovascularisation (Sanz). Dynamic Contrast Enhanced (DCE) Magnetic Resonance Imaging (MRI) is a well-known method for quantitatively imaging the kinetic properties of the vascular bed in many tissues. In application to atherosclerosis, DCE-MRI has been shown to identify plaques in the carotid arteries and correlate to neo-vascularisation identified in histology (Calcagno 1, Kerwin).

DCE-MRI involves detecting the change in MR signal induced by a contrast agent (CA) tracer injected in the bloodstream and imaging its course through the tissues. Accurate measurement of the kinetic parameters of traversal through the tissue bed requires measurement of the input function of contrast material in the arterial supply (AIF) as well as the signal change in the tissue (Ct).

Current DCE-MRI methods extract AIF and Ct information from the same image data. This is sub-optimal since the tissue should be imaged with high spatial resolution, shows only weak signal enhancement, and enhances over a slower time scale, whereas the AIF has opposite requirements. Dual imaging techniques (Calcagno 2, Kim, Gatehouse) acquire the AIF and Ct data in separate images, acquired either separately (with a low-dose AIF scan) or simultaneously (with the higher dose required for imaging uptake in the tissue). Simultaneous dual imaging techniques offer advantages: i) ease of scan protocol, ii) elimination of uncertainty of scaling CA concentration from different doses with potentially different characteristics of mixing in the vessels, and iii) elimination of conflict between spatio-temporal resolution requirements of AIF and tissue scans.

DCE-MRI in the human carotids poses particular challenges. The required high temporal resolution limits acquisition to a 2D slice. In the presence of high flow (20-90 cm/s), it is difficult to sustain steady state imaging as spins flow into the slice between every excitation. In dual imaging, where AIF bolus contrast agent concentration is high (0.1 mM/kg), T1 is very short (10 - 120 ms), and rapid acquisition is required to capture signal dependence on T1 before signal relaxation. This study investigates segmented and phase reordered turbo field echo (TFE) imaging as a strategy for imaging the AIF for dual imaging in the carotid arteries.

Methods & Results For the following imaging strategies: linearly phase-ordered turbo field echo (L-TFE), centric phase-ordered TFE (C-TFE), and n-segmented TFE (both linear and centric), modulation transfer functions (MTF) were calculated from modelling the signal evolution of inflowing blood-water spins over a range of contrast agent concentrations (i.e. T1 relaxation rates). Inflow was modelled as steady parabolic flow, with maximum speed 200 mm/s. Each MTF was applied to synthetic k-space of a model neck that included bright carotid arteries, and the resulting images were used to measure average vessel-ROI signal, which were then plotted against blood T1.

Simulated signal curves show consistency with AIF data acquired in vivo with linear and centric single shot acquisitions with differing CA doses (i.e. range of blood T1 during the bolus.)

In vivo, additional image artefacts may be caused by pulsatility in the carotid arteries, which should be included in future signal models.

Conclusion Simulation data indicates that linear phase-ordered, 4-fold segmented TFE, with a flip angle of 30° shows high signal sensitivity and sensitivity to changes in T1 during the bolus passage of the high-dose AIF used in dual imaging methods applied in the high-flow regime of carotid vessel imaging. Conversely, linear ordered, single-shot acquisition with low flip angles show the poorest sensitivity to changes in T1 during the bolus. Segmentation of the acquisition strategy incorporates additional saturation pulses that increase SAR and reduce temporal resolution, however, 4-fold segmentation increases AIF scan time by only 10% which is tolerable.

Clinical Relevance

Increased accuracy and robustness in measuring the AIF will allow improved estimation of kinetic parameters describing neovascularisation in the vessel wall in studies of atherosclerosis.



Figure 1: The dual imaging approach. Pre-saturation gives sensitivity to T1 recovery. Single shot low spatial resolution TFE acquisitions are acquired for the AIF, and

Table 1: Temporalresolution of the AIFchanges with segmentationstrategy due to increasedtime required to playsaturation pulses.

The 2nd Annual TMII Symposium-2011



interleaved with segmented high resolution TFE acquisitions for the tissue image. This strategy is referred to so as SHILO (Calcagno 2).

Segmentation	Number of shots (i.e.	Shot duration / Scan time
strategy	saturation pulses)	(320 x 80 matrix AIF image)
1-TFE (single shot)	1	535 ms / 535 ms
4-TFE	4	146 ms / 584 ms
8-TFE	8	101 ms / 808 ms
80-TFE	80 (one sat pulse per acquired line of k-space)	71 ms / 5680 ms





Figure 2: (Above) Example signal evolution curve, MTF, and image from a 4-segmented linear and centric acquisition. First and subsequent shots differ due to modulation from inflow. Centric acquisition shows typical image blurring from the MTF modulation. Acquisition parameters were: T1 = 216 ms, FA = 30, tfe-factor = 20, n-shots = 4.

Figure 3: (Left) Sensitivity to changes in T1 during bolus passage. Signal intensity plotted against T1 for a range of segmentation and phase ordering strategies.

Figure 4: (Below) In vivo AIF signal-time curves (dark blue lines) showing different signal behaviour as a function of T1 (dose) and imaging strategy for single shot linear phase ordering, a) low dose (0.01 mmol/kg, assumed bolus conc. range 0.2-2 mM, T1 range 120-670 ms), b) high dose (0.1 mmol/kg, 2-20 mM, 13-120 ms), c) intermediate dose (0.05 mmol/kg, 1-10 mM, 25-220 ms), and d) single shot centric ordering intermediate dose (0.05 mmol/kg, mM, ms).



References (Sanz) Nature 2008, (Calcagno 1) ATVB 2008, (Kerwin) MRM 2008, (Calcagno 2), (Kim) JMRI 2006, (Gatehouse) JMRI 2006



Statistics-Based Registration Applied to Dynamic Contrast-Enhanced Magnetic Resonance Images of Human Carotids

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Introduction

Dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) can be used the study inflammation in blood vessels with atherosclerotic plaque. In a DCE-MRI carotid vessel study, multiple frames of MR images are captured over time, and the signal intensity changes due to contrast-agent perfusion are analyzed. In order to accurately track the signal within the vessel wall, it is necessary for all the captured frames to be spatially aligned. However, due to patient motion the position of the vessel wall may have shifted from frame to frame resulting in inaccurate signal intensity curves. A registration method needs to be applied during the postprocessing phase to re-align the frames before proceeding with further analysis. In this study, we propose using statistics-based registration (C. M. Gallippi, Journal of Cardiovascular Magnetic Resonance, 2002) on DCE-MRI of human carotid arteries.

Methods

Statistics-based registration (SBR) was tested on DCE-MRI studies of the right and left carotid arteries of 5 patients. The images were acquired using a multi-slice black-blood turbo spin-echo (TSE) sequence with the following imaging parameters: 4 axial slices; TE, 8.27 ms; TR, 667ms; in-plane spatial resolution, 0.5x0.5mm; field of view, 160 mm², time resolution, 40s per frame; total scanning time, 13min 20 s, for a total of 20 frames. After acquisition of the first three frames, 0.1 mmol/Kg of Gd-DTPA was injected using a power injector, at a rate of 4 ml/s, followed by 20 ml saline chase. For each patient, both the right and left carotids in each of the four slices were treated as a dynamic series and analyzed separately. The images were cropped to a 64 x 64 pixel area surrounding the artery, and a single frame with good contrast about halfway through the dynamic series acquisition was selected as a template image. All the other frames in the series (called target images) were aligned to the template. Each target image was filtered with an edge-detection filter, and pixels with the highest edge information were designated as landmark pixels. Statistical metrics such as mean and standard deviation were computed in a small region-of-interest (ROI) surrounding each landmark pixel, and a search was performed to find a matching ROI in the template image that had comparable statistical values. The center of the matched ROI was saved as the new location of the landmark pixel. Once the new locations of all landmark pixels were determined, the target image was transformed to match the template image. The success of the alignment was judged visually by averaging all the frames of a series together before and after registration. The results were quantified by computing the correlation between each frame and the template image before and after registration. An average value of correlation per patient, per vessel was computed by averaging over all slices. A paired-samples t-test was used to compare average correlation values before and after registration.

Results

Figure 1(b) shows two consecutive frames overlaid before registration and Fig. 1(c) shows the same frames after registration, where the shift between the frames has been corrected. When all the frames in a DCE-MRI series are averaged together, the resultant image is blurry due to spatial misalignment, as shown in Fig. 2(a). However, once the SBR method is applied, the resultant average image is sharper, and the vessel wall boundaries are clearly visible. Figure 3 displays a box plot of mean correlation values over the entire sample set, computed before and after registration. The plot shows that there is a marked improvement in the correlation measure from un-registered to registered images. This was also reflected in the results of the paired t-test, which showed significant improvement (p < 0.001).

Conclusion

A statistics-based registration technique was evaluated for alignment of DCE-MRI studies of the human carotid arteries. The SBR method was found to be very effective at correcting the effects of motion on the dynamic series. The frames in the series were successfully aligned with a template frame chosen from the middle of the series. The improvement in the average correlation of a series after registration validated the performance of the SBR method for registration of DCE-MRI of carotid arteries.

Clinical Relevance

It is possible to characterize tissue by studying the uptake of contrast agent in the vessel wall with DCE-MRI. However, it is necessary to acquire accurate signal intensity curves by ensuring that individual frames of the dynamic series are properly aligned prior to further analysis. Registration is an essential pre-processing step that facilitates computing accurate results for future kinetic modelling.



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Figure 1: (a) Single frame from a DCE-MRI carotid artery study with the cropped regions shown in (b) and (c) marked by a red square (b) Two consecutive frames overlaid before registration. The arrow indicates an area of misalignment between frames. (c) The same two frames after registration showing the correct alignment of the vessel wall.

Figure 2: (a) All frames of the DCE-MRI series averaged together prior to registration. (b) All frames averaged after registration. The wall of the vessel is now clearly delineated showing the effects of registration.









SHILO: Simultaneous High/Low spatial/temporal resolution dual-imaging acquisition for improved parameters quantification in dynamic contrast enhanced (DCE) MRI of atherosclerosis

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Introduction Dynamic contrast enhanced (DCE) MRI has recently been applied to plaques' neovessels, a hallmark of high-risk vulnerable lesions (*Calcagno C et al, ATVB, 2008, Kerwin WS et al, MRM 2008*). The accuracy of DCE-MRI measurements depends on accurate sampling of both arterial input function (AIF, the concentration of contrast agent in the blood plasma) and tissues enhancement curve. These two compartments have very different requirements in terms of spatio-temporal resolution and signal range(**Table 1**). Therefore, accurate sampling of both compartments would require using a different set of imaging parameters, whic cannot be achieved with conventional imaging sequences if both have to be acquired simultaneously. In this abstract we present a new dual-imaging sequence, **SHILO** (Simultaneous **High Low**, to simultaneously acquire both the vessel wall and AIF curves with tailored imaging parameters.

Methods & Results: Building upon the work of Kim et al. (Kim D et al, [MRI 2006) in myocardial perfusion, we modified a spoiled, multi-shot, saturation prepared turbo field echo (TFE) sequence into a dual-imaging sequence for DCE-MRI of atherosclerosis. In our scheme, we acquire a low spatial/high temporal resolution single-shot AIF image ("LO") and simultaneously a high spatial/low temporal resolution multi-shot vessel wall image ("HI") (Figure 1). If a saturation pulse is applied before the first excitation of each shot, and the saturation delay before acquisition of **HI** is longer than the **LO** acquisition time, then the **LO** image can be acquired between the saturation pulse and the beginning of the **HI** image acquisition (**Figure 1**). By inserting the acquisition of the LO image after the saturation pulse, but before the acquisition of the HI image, it is possible to acquire two images with different dynamic signal range, different spatial and temporal resolution within the same acquisition. The different dynamic signal range derives from the shorter (LO, AIF) and longer (HI, vessel wall) delay after the saturation pulse. The different spatial resolution derives from different number of k-space lines acquired (single shot, LO image; multi-shot, HI image). In addition, for every HI (vessel wall) image, several LO (AIF) images will be acquired, with an acceleration factor determined by the number of shots necessary to acquire the HI image. *Image Acquisition*: Two atherosclerotic rabbits and two subjects with carotid artery disease were imaged for preliminary testing on a 3T clinical system, using a conventional knee coil for rabbits and a dedicated 8-channel carotid coil for the human carotids. SHILO DCE-MRI was performed on one selected axial slice (imaging parameters: TR, 9.6 ms; TE, 2.4 ms; flip angle 12 degrees; FOV, 16X16cm; matrix size, 320X320; spatial resolution, 1 mm in plane for LO, 0.5 mm in plane for HI; echo train length, 80; 4 shots). Time resolution for LO was \sim 1s, while for HI was \sim 4s. *Image* Analysis: A linear correlation was assumed between signal intensity and contrast agent concentration. Kinetic modeling was performed with a modified Tofts model. Figure 2 and 3 show examples of anatomical images, parameters maps and time curves from one atherosclerotic rabbit acquired with SHILO.

Conclusion: In this preliminary study, we successfully demonstrated the use of **SHILO** for accurate AIF sampling, and adequate spatial resolution images for vessel wall acquisition in both atherosclerotic rabbits and human subjects.

Clinical Relevance: SHILO allows sampling the AIF and tissue curves more accurately than traditional acquisitions. This may have significant impact on the reliability of kinetic parameters estimation and may reflect positively on the ability of DCE-MRI to assess severity of disease or to track changes in neovascularisation due to therapeutic intervention. Despite being conceived to more accurately quantify neovessels in atherosclerosis, this same acquisition scheme can be applied to any perfusion/permeability studies to improve parameters estimation.



Figures and tables

Table 1	AIF	Vessel Wall
Spatial Resolution	Low	High
Time Resolution	Fast	Slow
Slice Coverage	Limited	Extensive
Concentration	High	Low



Figure 1: SHILO sequence diagram. The time resolution of LO is 4 times faster than the one HI, that however have 4 times higher spatial resolution.





Figure 2: Kinetic parameters maps of aortic vessel wall calculated from SHILO T2W acquisition. (A) image for anatomical reference, (B)K_{ep} map. (C) K^{trans} map. (D) v_p map.L: vessel lumen. (E) Fused anatomical T2W, with color coded K^{trans} (green) and v_p (red) maps(full K: kidneys,. SM: skeletal view). muscle.(F) Zoomed in view of E. Lumen is colored black or yellow for better clarity.

Figure 3: Signal intensity curves from SHILO. Red, AIF (LO). Blue, Vessel wall (HI). Green, Skeletal muscle (HI). Black, AIF gamma variate fit. x axis, time (min). y axis, signal intensity





Development of a new diagnostic tool for myocardial fibrosis using multimodal imaging nanoparticles in an animal model of heart failure.

Authors & Affiliations

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Introduction

Advances in myocardial molecular imaging have led to the beginning of biomarkers acceptance in the assessment of several cardiac disease processes such as myocardial fibrosis. Many of the biomarkers currently available remain at the level of clinical and preclinical research and will require additional clinical investigation before they can be considered as part of routine standard care for cardiac patients.

Study design and methodology

<u>Study design</u>: This study will take place in a rat model of congestive heart failure. Animals will be injected intravenously with gadolinium lipid micelles containing a target for collagen (EP-3600) or scramble peptide (EP-3612) and a near infrared (NIR) dye in order to detect *in vivo* the presence of fibrosis by magnetic resonance (MRI) and NIR imaging techniques. The MRI will be also used to assess the heart functional parameters of these rats. Finally, after the imaging study the animals will be sacrificed and histological and molecular studies of myocardial fibrosis will be used to corroborate the results obtained by imaging techniques (Figure A).

Synthesis and characterization of collagen-targeted multimodal micelles: Collagen-targeted micelles will be prepared by the lipid film hydration method. Micelles will be synthesized with Gadolinium-DTPA, DSPE-PEG2000, DSPE-PEG2000-COOH, cholesterol, Rhodamine-PE and NIR dye (DiR) in a molar ratio of 10:8:2:9:0.2:0.6. After hydration, micelles will be modified with EDC-NHS in a molar ratio of 20:10:1 DSPE-PEG2000-COOH:EDC:NHS and incubated with a collagen-targeted peptide (EP-3600) or a scramble peptide (EP-3612) that include a reactive amine group in a 1:1.1 DSPE-PEG-COOH:EP-3600 or EP-3612 molar ratio (Figure B, collagen-targeted micelles). Finally, micelles will be characterized for mean hydrodynamic diameter, relaxivity and protein concentration.

<u>In vitro collagen binding assay</u>: Micelles will be incubated with collagen type I in 96-well plates in order to test the binding of EP-3600 micelles with collagen. The fluorescence intensities of rhodamine and DiR will be measured in a microplate reader and in a NIR scan respectively.

<u>Animal model of congestive heart failure</u>: Rats will undergo aortic constriction to introduce hypertrophy, and two months later, coronary arterial ligation to induce ischemic injury and accelerate congestive heart failure. During the third month the aortic binding will be release and finally rats will be sacrifice 4 months after the first surgery.

<u>Cardiac magnetic resonance</u>: Imaging studies will be performed at the beginning, at 8, 12 and 16 weeks with a Philips 3T Achieva scanner. Collagen-targeted micelles will be injected intravenously 24h before the scan. A knee coil and a small animal gating system will be used. (Figure C, MRI sequences).

<u>Near infrared imaging</u>: NIR imaging studies will take place afterwards MRI imaging in the Fluorescence-Assisted Resection and Exploration (FLARE) large animal, single-channel intraoperative NIR fluorescence imaging system. Images will be acquired on two 12-bit Orca-AG cameras used within their linear range and after computer-controlled camera acquisition, standard (white light) and 2 functional (NIR fluorescent light) video images can be displayed separately and merged (Figure D).


<u>Ex vivo</u> fluorescence collagen imaging: After sacrifice, rhodamine and NIR fluorescence imaging in myocardial tissue slides will be studied by microscopy.

<u>Histological and molecular studies</u>: Picrosirius red staining and immunohistochemistry of collagen type I and II will be performed to quantify myocardial fibrosis. Colorimetric techniques will be used to assess collagen crosslinking.

Collagen synthesis and expression of involved enzymes will be analyzed by PCR and western blot. Blood samples will be used to quantify pro-collagen type I carboxy-terminal proteinase (PICP) levels by ELISA.

Clinical Relevance

Whereas circulating biochemical markers may offer many logistics advantages, they may not prove as sensitive as imaging markers in the molecular detection or assessment of cardiac disease processes such as myocardial fibrosis. Combination of biochemical and imaging markers might have inherent advantages for developing new diagnostic tools in the assessment of cardiac disease by non-invasive techniques.

Figures and tables



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Development of comprehensive 3D evaluation of atherosclerosis in multiple vascular beds

Authors & Affiliations

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Introduction

Since atherosclerosis is a systemic disease that affects all vascular beds[1], MRI can be useful in monitoring its outcomes throughout the whole body. 3D dark blood MRI is quickly becoming the preferred methodology for evaluating atherosclerotic plaque burden non-invasively [2]. It is also becoming more common to use such imaging approaches in multiple vascular beds including the aorta [3], carotids and femoral arteries [4] in an attempt to evaluate systemic disease burden instead of focusing on specific lesions. Such approaches can then be used to evaluate changes in burden in response to treatment and/or be used to evaluate progression or regression of disease over time [5]. 3D approaches are often preferred to 2D approaches because of increased SNR obtained and due to the minimization of volume averaging artifacts from 3D scans. The ability to obtain isotropic voxels thereby enabling multi planar reformatting of images is yet another benefit of 3D approaches. The most promising 3D black blood approach currently being investigated is the variable flip angle TSE (SPACE) approach [6]. Before this technique can become clinically applicable, however, its robustness must be established. For this purpose, it is increasingly important to develop a patient positioning and protocol implementation workflow for optimum reproducibility of imaging results. Here we propose the use of the 3D SPACE sequence to evaluate the carotids, aorta and superficial femoral arteries in patients at risk for cardiovascular disease using a consolidated imaging approach in the same imaging session.

Methods and Results: 15 patients at risk for cardiovascular or atherosclerotic disease were scanned using both 3D SPACE and conventional 2D multi contrast TSE imaging. All MR images were obtained on a 1.5T Siemens whole body MR imaging system. The scans were divided into 3 segments for appropriate anatomical coverage. First, the carotids were scanned extending 3 cm below and above the carotid bifurcations using a 4-channel carotid coil. Secondly, the entire length of the aorta from the aortic arch to the iliac bifurcation was scanned using the spine array and two body matrix coils. Finally, the iliacs and the bilateral superficial femoral artery were also imaged using the spine array and body matrix coil. Imaging was performed using a 3D cardiac gated scan with navigator control for respiratory gating SPACE sequence for the aortic images and using a non-cardiac and respiratory gated SPACE scan for carotids and femoral arteries. Image resolution was approximately 1.1mm isotropic voxel for the aorta and approximately 0.8mm isotropic voxel size for the carotids and approximately 1mm isotropic voxel size for femoral imaging. 12 slice 2D TSE images using multi-contrast weightings and using parallel saturations bands for flow suppression were also obtained for all three vascular beds for comparison purposes. The in -plane resolution for 2D scans was 0.7mm for aorta and 0.5mm for carotids and femorals. Images were subjectively evaluated for 4 distinct criteria (overall image clarity, vessel wall delineation, flow suppression and artifacts) using a 5 point scale ranging from 1-5 with 1 being poor and 5 being excellent [8]. A Mann-Whitney rank sum test was used to compare the scores obtained using the qualitative analysis. A p-value < 0.05 was considered significant. Total imaging time was < 45 minutes for all three vascular beds combined for the 3D scans. The 2D scans also took 45 minutes to cover the same vascular territory.

Images using 2D TSE and 3D SPACE were successfully acquired from all15 subjects for all three vascular beds. Sample images obtained are shown in Figure 1.The qualitative analysis results are presented in Table 1. Results indicated that for carotids and aorta, there was no significant difference between the 2D TSE and SPACE approaches qualitatively. For femoral imaging however, the SPACE sequence fared more poorly in all criteria indicating that the current protocols for femoral imaging needs improvement to be compared to traditional 2D imaging. This might have to do with the smaller size of the femoral arteries and poorer resolution in plane of the SPACE sequence or lack of specialized coils for femoral imaging. Partial voluming effects were however higher in the 2D images and therefore plaque burden estimation using 3D SPACE is expected to be more robust.

Conclusions: 3D SPACE appears to be an excellent imaging approach to determine overall plaque burden in multiple vascular beds in the same imaging session. Subjective image quality is comparable to 2D TSE approached while providing advantages of more accurate burden measurements and improved vessel wall coverage with same scan time.

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Clinical Relevance

This abstract proposes a workflow for accurate 3D imaging of atherosclerosis in multiple vascular beds. This work paves the way forward for moving the field of atherosclerotic plaque characterization from research to clinical practice by proving a robust and reproducible approach.

Figures and tables

Table 1: Mean and SD of Qualitative Assessments							
Vessel	Sequence	ImQ	VWD	FS	Artifacts		
Carotids	2D TSE	4.0 ± 0.67	4.3 ± 0.67	4.2 ± 0.63	4.1 ± 0.73		
Carotids	SPACE	3.7 ± 0.67	4.2 ± 0.78	4.5 ± 0.52	3.9 ± 0.73		
Aorta	2D TSE	3.9 ± 0.73	4.0 ± 0.67	3.8 ± 0.63	3.4 ± 0.84		
Aorta	SPACE	4.0 ± 0.67	4.3 ± 0.82	4.1 ± 0.56	3.6 ± 0.84		
Femoral	2D TSE	3.9 ± 0.73	3.4 ± 0.84	3.5 ± 0.85	4.0 ± 0.67		
Femoral	SPACE	2.8 ± 0.79*	2.3 ± 0.94*	2.4 ± 0.96*	3.1 ± 0.57*		
ImQ: image quality; VWD: vessel wall delineation; FS: flow suppression; * indicates							



Abstracts, Neuro Imaging category





3D Quantitative Micro-MRI Mapping of Alzheimer's Plaques in Transgenic Mice using A β 1-42 Targeted-USPIOs

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Introduction

The visualization of Amyloid β (A β) plaques in the brain is very important to monitor Alzheimer's disease (AD) progression and to evaluate the efficacy of therapeutic interventions. Only two groups have been so far successful in enhancing A β plaques using targeted contrast agent in transgenic mice [1,2] using intra-carotid injection in conjunction with mannitol to increase their permeation across the blood brain barrier (BBB). In the present study, we examined whether the use of ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles, chemically coupled with A β 1-42 peptide along with mannitol through femoral intravenous injection can be effective

Methods & Results

Animals: 13 to 17-month old APP/PS1 transgenic mice [4] and age-matched wild-type (C57Bl/6J) control mice were used in these studies. Targeted contrast agents: The USPIO nanoparticles (10mgFe/ml, Ocean Nanotech) were linked to A β 1-42 peptide using standard EDC/NHS coupling methods, according to the manufacturer's instructions. MRI: All MRI scans were performed on a 7T micro-MRI system. A 3D multi-Gradient Echo sequence was used to provide quantitative R2* measurements. The third echo was used to generate a T2*-weighted (TE=12.3 ms) image subset for both plaque visualization and voxel-based analysis [3]. Histological Studies: After ex vivo MRI, serial coronal sections (40 µm) were cut and every third section were stained with monoclonal anti-A β antibodies as previously described [1]. Voxel-based morphometry (VBM): was performed as an additional quantitative analysis based on SPM5 (Wellcome Dept of Clinical Neurology, London) with SPMMouse toolbox [5]. Regional specific differences were assessed statistically using the GLM/univariate analysis with a one-tailed T statistics, showing voxels of lower intensity in APP/PS1 Tg mice compared to wild-type mice where p <0.01 (uncorrected for multiple comparisons) indicated statistical significance for individual voxels, with a minimum cluster size of 500 voxels.

Fig (A) depicts an example of *in vivo* T2*-weighted MRI of a 14 month-old APP/PS1 mouse showing multiple dark spots throughout the brain, 6-h following USPIO-A β 1-42 injection. These dark enhancements closely match the larger plaques (arrowheads) confirmed by immunohistochemistry (B). The regional differences seen in VBM comparing USPIO-A β 1-42 injected APP/PS1 and WT mice correlated (C-H) with the plaque distribution observed histologically, contrasting with no differences between the two groups of mice without contrast agent (data not shown). Furthermore, R2* quantitation assessed in various brain regions between APP/PS1 Tg mice and wild-type control showed significant differences in the cortex and the hippocampus (** p<0.01) but not in the cerebellum as expected in this mouse model.

Conclusion

Our results demonstrate that USPIO-based A β probes can help visualize individual large plaques after femoral injection. Combination of our targeting approach with quantitative methods help identify statistically differences between AD transgenic mice and wild type mice. The feasibility of using less invasive intravenous femoral injections for A β plaque detection in AD transgenic mice facilitates using this method for longitudinal studies in the pathogenesis of AD.

<u>Acknowledgments</u>: We thank S.J. Sawiak for providing the SPM mouse brain atlas. This work was supported by NIH grants AG20245, NS073502 and AG008051 to T.W., the American Health Assistance Foundation ADR (A2008-155) to Y.Z.W. and the Alzheimer Association (IIRG-08-91618) to Y.Z.W.



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Clinical Relevance

One paragraph of 2-4 sentences

Visualization of amyloid b plaques in the brain of living mice is very important to monitor Alzheimer's Disease progression and to evaluate the efficacy of therapeutic interventions.

Figures and tables





Design and Characterization of A Set Of MRI Histology RF Coils Dedicated to Standardized Slide Sections D. M. Hoang1, C. Zhang1, M. Shamsie1, L. Fakri-Bouchet2, and Y. Z. Wadghiri1

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Introduction

Preclinical studies predominantly rely on the use mice as subjects to test and validate novel MRI techniques. One of the major challenges encountered in validating the MRI findings is the lack of accurate co-registration with histology. In the present study, we designed and tested a set of three histological coils that permits direct MR imaging of histological tissue samples [1,2]. Several mouse organ were examined spanning from the olfactory bulb to the liver, one of the largest organ.

Methods & Results

Tissue sectioning and preparation: Histology slices of different mouse organs were sectioned with slice thickness ranging from 30µm to 60µm and were conserved in Cryo-Protectant. Each tissue section was hydrated by immersion in either a buffer solution or a buffer doped with 5mM Gd-DTPA [3] both degassed using a vacuum chamber for 30-min to prevent presence of air bubbles. The slice was subsequently mounted on a #1 glass cover-slips (glass thickness for each \sim 130-170µm) surrounded with hydrophobic Fomblin (Solvay Solexis Inc., Thorofare, NJ) to prevent dehydration and then sandwiched with a second cover-slip resulting in an overall thickness that should be less than 400-µm for a tissue section reaching 60µm thickness. The physical dimensions of the three coils were then designed accordingly to fit optimally any of the cover-slip as summarized in Table 1. MRI: All experiments were performed on a 7-T Bruker micro-MRI system. Unless noted otherwise, all the acquisition parameters of the MRI sequence were as follow: 2D single slice Multi-Gradient Echo (8 echoes, TE: 3.2-ms, ES: 5.2-ms), TR: 300-ms, Flip Angle was adjusted empirically to maximize SNR (Ernst Angle) in each experiment depending on the sample preparation. Both matrix and FOV were varied depending on the dimension of the samples leading to an inplane resolution ranging from 50- μ m to 60- μ m The bandwidth was maintained constante (293Hz/pixel). The number of averages was chosen to keep the SNR higher than 30 (Mean Signal[sample]/Std Dev.[background noise]) with scanning time ranging from 1 to 8 hours.

Based on the effective rf field volume estimated from the dimension of our coils, a 3x increase in sensitivity was expected for the small coil (SHC) and 1.5x for the medium coil (MHC) both relative to the large coil (LHC). This was inferred from the expected improvement in filling factor. Experimental measurement using an identical tissue sample for the three coils were as follow: 3.6x for the SHC and 1.3x for the MHC both relative to the LHC. Figure 1 illustrates sections obtained from various mouse organs with corresponding histology section using a flat bed scanner. MRI and corresponding optical microscopy examples were all obtained from fresh fixed tissue section immersed in buffer solution without any prior tissue staining, unless noted otherwise.

Conclusion

Large tissue samples (FOV=20-mmx40-mm) can be imaged in less than 8 hours with a minimum 60- μ m section (in-plane: 60- μ m). Similarly, tissues that can fit a 10x8mm FOV could be acquired in less than 2-hours. Doping the buffer solution with 5-mM GdDTPA leads to 2.4x gain in SNR enabling the acquisition of tissue section as thin as 10-um thickness in 8-hours using our smallest histology coil.

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Clinical Relevance

This work helps address one of the major challenges encountered in validating the MRI findings through accurate co-registration with histology by direct imaging of histological tissue samples.

Figures and tables

Table 1

Coil	Physical Dimension of the probe LxWxD (mm)	Effective volume of the radiating RF within the coil (mm ³)	Accessible Volume by the cover-slips LxWxD (mm)	Dimension of Effective RF field accessible by sample LxWxD (mm)	Reference and dimension of standard Cover-slip #1 LxW (mm)	Mouse Organs	Matrix
Small	28.0x10.5x0.6	117.6	14.0x10.5x0.4	10.0x8.0x 0.1	Gold Seal [®] (ref.:3312) 24x12	olfactory bulb, eyeball, spinal cord, etc	256x256
Medium	40.0x26.0x0.6	416.0	26.0x26.0x0.4	20.0x20.0x0.1	Fisherfinest [®] Premium (ref.:12-548-5M)	brain's cortex, kidney, spleen, heart	256x256
Large	50.0x47.0x0.6	940.0	26.0x47.0x0.4	20.0x43.0x0.1	24x50	Liver, Lung	512×1024





New structure preserving denoising technique produces better quality images than Adaptive Statistical Iterative Reconstruction with the same Low Radiation Dose

Authors & Affiliations

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Introduction

The low radiation doses increasingly utilized for CT have high levels of noise when reconstructed with filtered back projection (FBP). Iterative reconstruction, including adaptive statistical iterative reconstruction (ASIR), is an alternative computationally intense reconstruction technique that reduces image noise and improves low contrast detectability. Another method of noise reduction is structure preserving denoising (SPD) which can be applied to any CT image of any generation or manufacturer. We evaluated quantitative and qualitative measures of dose reduced CT studies of the head reconstructed with either FBP or ASIR, and FBP images processed with SPD (FBP-SPD).

Methods & Results

19 head CT examinations performed using a reduced radiation dose (40% lower than had been used before ASIR introduction) were processed using both FBP and ASIR. A novel non-isotropic 3D edgesensitive algorithm was applied to the FBP data set (FBP-SPD). Signal-to-noise (SNR) and contrast-tonoise ratios (CNR) for the 3 image datasets were calculated from regions of interest in the basal ganglia and centrum semiovale. Qualitative assessment of each study for overall quality and greywhite distinction was performed on a 4-point scale (1-best, 4-worst) by two radiologists blinded to the reconstruction method.

Compared to dose reduced FBP, SNR increased 31% and CNR increased 29% with the application of SPD to FBP images. ASIR increased SNR 15% and CNR 21%. The neuroradiologists scored grey-white distinction at 2.5 \pm 0.6 for FBP, 1.8 \pm 0.4 for FBP-SPD and 1.6 \pm 0.5 for ASIR. Overall quality was scored at 2.4 \pm 0.5 for FBP, 1.5 \pm 0.50 for FBP-SPD and 1.6 \pm 0.5 for ASIR. The scores for both grey-white distinction and overall quality for the ASIR and FBP-SPD images were statistically significantly better than the scores for the FBP images (p=0.0001) but there was no statistically significant difference between the scores for the ASIR and SPD images.

Conclusion

Structure preserving denoising (SPD) of low dose head CT data produces images markedly better than those reconstructed with conventional FBP and comparable in quality and grey-white distinction to those produced using ASIR.

Clinical Relevance

SPD is manufacturer agnostic technique that could permit reduced dose CT imaging throughout an imaging enterprise without a reduction in image quality.



Figures and tables





Resting State Functional Connectivity in Blast-related Mild Traumatic Brain Injury in OIF/OEF Combat Veterans with and without PTSD

Authors & Affiliations

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Introduction

Imaging studies of blast-exposure mild traumatic brain injury (mTBl_B) in veterans of the Iraq and Afghanistan conflicts are now emerging. Functional connectivity magnetic resonance imaging (fMRI) is a technique that analyzes the functional relatedness of select brain regions that demonstrate temporally correlated blood oxygenation level-dependent (BOLD). fMRI studies have been used to investigate Default Mode Network (DMN) activity, which is an interconnected and anatomically defined brain system in which signals are higher while a subject is at wakeful rest and are lower when a subject is engaged in a cognitive activity. Studies of the DMN in civilian mild TBI demonstrate decreased functional connectivity within the DMN following attention or working memory tasks. To date, no studies have investigated functional connectivity and the DMN in combat veterans with and without blast-related mild TBI.

Methods & Results

Participants were Operation Iraqi Freedom/Operation Enduring Freedom (OIF/OEF) veterans with mTBI_B (N=14) and 4 OIF/OEF healthy, combat controls (HC), who were matched on age and education **(Table 1).** The mTBI_B group was comprised of volunteers who had been evaluated at the JJP VAMC Polytrauma/Traumatic Brain Injury Unit and met mTBI_B diagnosis, as per VA and standard clinical guidelines. Combat controls were OIF/OEF veterans who did not have a diagnosis of mTBI_B. Exclusion criteria for all subjects were any significant medical illness, current substance use/abuse, major psychiatric illness, history of TBI prior to military service, or any ferrous material that would prohibit MRI scanning. All were screened for PTSD either through clinician interview or through questionnaires to gauge PTSD symptomatology. A total of 11 subjects screened positive, or had a diagnosis of PTSD (**Table 1**).

Imaging was done at Mount Sinai School of Medicine, Department of Radiology on a 3T Siemens Allegra head dedicated MRI system. The fMRI protocol consisted of two 4 minute resting scans. Impaired cortical function can be assessed using functional connectivity resting fMRI data and an anatomically defined seed cluster.

Two analytical approaches were used for analyzing the resting state fMRI data. For the first analysis, the DMN was detected in resting state fMRI using an independent component analysis (MELODIC; Beckmann 2004). The DMN includes four distinct regions: the anterior cingulate cortex (ACC), the posterior cingulate cortex (PCC), the right parietal lobe (RP), and the left parietal lobe (LP). Dual regression was used to compare the DMN between patients with mTBI_B and combat controls throughout the whole brain in a voxel-wise manner (courtesy of C. F. Beckmann). A voxelwise t-test showed that veterans diagnosed with mTBI_B, exhibited significantly higher coactivation (**Figure 1** areas highlighted in blue) in the PCC, RP and LP, and lower coactivation (**Figure 1**; p < 0.05; uncorrected for multiple comparisons).

The second resting state fMRI analysis quantified functional connectivity between the major components of the DMN. Functional connectivity was analyzed for the following regions: PCC-to-RP, PCC-to-LP, ACC-to-PCC, and RP-to-LP (**Figure 2** blue areas indicate significant functional connectivity). A two-tailed unpaired t-test was used to compare functional connectivity of the mTBl_B sample to the combat controls. The results showed that the functional connectivity between PCC-to-RP (t=-2.58, p=0.020), as well as the PCC-to-LP were significantly sronger in veterans with mTBl_B than in the combat controls (t=-2.25, p=0.039; **Table 2**).

The data were then submitted to a Multivariate Analysis of Covariance (MANCOVA), using the mTBI_B group (n=14) and the combat controls (n=4) as the between subjects variable, the functional connectivity between PCC-to-RP, PCC-to-LP, ACC-to-PCC, and RP-to-LP as the dependent measures, and PTSD as a covariate. Results indicated a trend for higher functional connectivity in mTBI_B as compared to combat controls between the PCC-to-LP (F=3.93, p=0.066) and the PCC-to-RP (F=3.81, p=0.070), such that mTBI_B had higher functional connectivity in combat controls (**Table 3**). Although not significant, mean/SD activation estimates **in all regions** were higher in mTBI_B as compared to combat controls (**Table 3**). When

MANOVA was conducted removing PTSD as a covariate, these same regions were significant (PCC-to-LP F=5.08, p=0.039, and the PCC-to-RP connection F=6.66, p=0.020; data not shown).



Conclusion

This is the first study of its kind in blast-related mild TBI in OIF/OEF veterans. Our findings demonstrate that veterans with blast-related mild TBI have higher levels of resting state coactivation compared to controls in three structures of the DMN: the PCC, RP, and LP regions. Furthermore, the functional connectivity between the PCC to the RP, as well as the PCC to the LP was higher in veterans with mTBI_B than in combat control veterans. These findings may indicate a dysregulated DMN in the patient group. The lower coactivation in the DLPC may have implications for the high rate of neurobehavioral problems in patients with blast-related mild TBI. The relevance of these findings for military personnel exposed to blast wave and their impact on clinical data and behavioral and cognitive function will be important areas for future investigations.

Clinical Relevance

This research will advance scientific knowledge by contributing to the understanding of neural damage in blastrelated TBI in returning OIF/OEF combat veterans. These findings support the hypothesis that blast-related mild TBI is a neurobiologically distinct entity, disimilar from PTSD. The findings will further knowledge of the clinical, behavior and cognitive profile in these cohorts and how brain connectivity can be altered due to blast exposure mTBI in military personnel.

Figures and tables



Figure 1 Figure 2. Table 1: Subject Demographics

	Combat Controls (n=4) Mean±SD	mTBl₅group (n=14) Mean±SD	F	p
Age	34.75±7.72	32.79±7.22	.224	.642
Education	14.75±1.89	13.5±1.91	1.34	.265
Years post-deployment	3.88±2.46	4.36±1.45	.255	.621
Sex	All Male	All Male	n/a	n/a
Handedness	1Left/3Right	1Left/13Right	n/a	n/a
PTSD	1	10	n/a	n/a

Table 2: Functional Connectivity Results

Region	t	р
ACC-to- PCC	-0.69	0.500
PCC-to-LP	-2.25	0.039
LP-to-RP	-1.18	0.255
PCC-to-RP	-2.58	0.020

Table 3: MANCOVA Results

Region	Group	Mean(SD)	F	р
ACC-to- PCC	HC	.796±.079	0.92	0.352
	mTBl _₿	.845±.133		
PCC-to-LP	HC	.710±.048	3.93	0.066
	mTBl _₿	.972±.227		
LP-to-RP	HC	1.10±.140	0.71	0.410
	mTBl₅	1.27±.273		
PCC-to-RP	HC	.654±.102	3.81	0.070
	mTBI₀	.883±.166		



Regionally Specific White Matter Abnormalities in OIF/OEF Combat Veterans With Blast-Related Mild Traumatic Brain Injury and PTSD

Authors & Affiliations

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Introduction

Imaging studies of blast-exposure mild traumatic brain injury (mTBI_B) in veterans of the Iraq and Afghanistan conflicts are now emerging. Studies have reported increased, decreased, or non-significant differences between veterans with mTBI_B compared with military or healthy civilian (HC) controls. We examined diffusion tensor imaging (DTI) fractional anisotropy (FA) in white matter underlying Brodmann areas (BAs) within the prefrontal and temporal cortices, and in the cingulum. DTI is a magnetic resonance imaging (MRI) technique used to examine the integrity of white-matter tracts by measuring the diffusion characteristics of water molecules. In white matter, FA is an index of the preferential diffusion of water parallel to the main fiber direction.

Methods & Results

Participants were veterans with $mTBI_B$ and healthy controls (HCs; Table 1). The $mTBI_B$ group was screened by a multidisciplinary team at the JJP VAMC Polytrauma Clinic to meet $mTBI_B$ diagnosis as per VA guidelines. The HC group was screened by a clinician for any history of head trauma, medical illness, Axis I or II disorder (SCID).

Imaging was done at Mount Sinai on a 3T Siemens Allegra head dedicated MRI system. We used our semi-automated parcellation technique based on the Perry post-mortem brain atlas to estimate the Brodmann areas (BAs). The program divides manually traced coronal MRI brain slices into 20 radial and 10 midline sectors in each hemisphere, and each temporal lobe into 16 sectors. These sectors are then assigned to 39 BAs.

Multivariate ANOVAs examined between-group differences in white matter FA in prefrontal cortex: anterior (BA 8,9,10), medial (BA 32, 24,25), orbitofrontal (BA 11,12,47) and dorsolateral (BA 44,45,46); temporal lobe: (BA 22, 21, 20); and cingulum (BA 25,24,31,23, 29) in each hemisphere.

A significant Group x Region x BA x Hemisphere interaction (F[6,120]=3.37, p=0.004, Wilks; Figure 1) indicated mTBI_B participants had higher FA in BA10 and lower FA in BA32 compared with HC in the left hemisphere (p<0.05, Fisher's LSD Tests). Conversely, in the right hemisphere, FA in BA10 was lower in the mTBI_B compared with HC group and increased in BA25 (all p<0.05). This interaction remained significant using PTSD as a covariate (p=0.024). Between-group differences were not significant in the temporal lobe and cingulum.

Conclusion

We demonstrate a complex interaction for white matter integrity that is regionally specific to anterior and medial prefrontal cortex and anterior cingulum. A region-of-interest approach may be important in analyses of brain trauma due to blast exposure. Further, our findings suggest that the white matter abnormalities observed are independent of comorbid PTSD.

Clinical Relevance

There are white matter deficiencies in combat veterans with blast-related mild TBI not accounted for by a diagnosis of PTSD. Using DTI may be helpful in identifying injury and brain pathways impacted in blast-related mild TBI. DTI and other forms of neuroimaging of specific biomarkers are valuable tools in furthering our understanding of mild TBI in our returning veterans from Iraq and Afgahnistan.



Figures and tables



FIGURE 1. Top shows left hemisphere and **Bottom** shows right hemisphere fractional anisotropy: mTBI_B participants vs. civilian controls x Region x BA x Hemisphere interaction, F[6,120] = 3.37, p = 0.00417, Wilks. *p < 0.05, post-hoc Fisher's LSD test.

	Healthy Controls (n=9)	Blast mTBI participants (n=13)
Age	Mean±SD 28.6±6.95	Mean±SD 32.1±7.0
Sex	2F/7M	All Male
Handedness	Right	Right
Yrs. post-deployment	N/A	4.3±1.5

TABLE 1. Sample Demographics:



White Matter Connectivity Abnormalities in Posttraumatic Stress Disorder

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Introduction

Post-traumatic stress disorder (PTSD) is a serious public health problem, especially among combat veterans: lifetime prevalence of PTSD is 2-3 times higher in combat veterans compared to the general population. PTSD has been well-characterized clinically as a cluster of hyperarousal, re-experiencing, numbing and avoidance symptoms; however, the underlying neurology of the disorder remains poorly understood. Neuroimaging investigations of PTSD have mainly focused on volumetric and functional differences but relatively little attention has been paid to white matter fiber correlates of the disorder. White matter fiber bundles that connect brain regions implicated in PTSD can be imaged using diffusion tensor imaging (DTI).

Methods & Results

The sample consisted of 20 Gulf War veterans with (n = 12) and without (n = 8) PTSD.

All imaging was performed on an Allegra 3T head-dedicated system (Siemens, Ehrlangen, Germany). DTI was acquired with a pulsed-gradient spin-echo sequence with EPI (TR = 4100 ms, TE = 80 ms, FOV = 21 cm, matrix = 128 × 128, 28 slices, thickness = 3 mm skip 1 mm, b-factor = 1250 s/mm2, 12 gradient directions, 5 averages).

DTI images were compared using two approaches: voxel-wise statistics and DTI-tractography. Voxel-wise analysis was performed using the tract-based spatial statistics (TBSS) routines implemented in FSL to compare fractional anisotropy (FA) between groups on the white matter skeleton image. Brute-force streamline DTI tractography was implemented in Matlab and used to quantify diffusion parameters of the cingulum bundle and the anterior limb of the internal capsule.

The exploratory whole brain white matter comparison revealed a trend towards lower mean diffusivity (MD) in the right anterior limb of the internal capsule in veterans with PTSD (p < 0.10; FWE corrected for multiple comparisons; Figure 1). No differences or trends in FA were detected.

A repeated measures MANOVA of the diffusion parameters from the DTI tractography showed a between-subjects effect of PTSD on MD (F(1,18) = 6.429, p = 0.021). Significance was not found with FA. Post-hoc t-tests revealed that this effect was driven by a lower MD in patients in the right cingulum bundle (t = 2.2, p = 0.39) and right anterior internal capsule (t = 1.9, p = 0.75) and, to a lesser degree, by the left cingulum bundle (t = 1.2, p = 0.23), and not by the left anterior internal capsule (t = -0.6, p = 0.56) (Figure 3).

Conclusion

A large body of the neuroimaging research on PTSD suggests that there is abnormal function of the fronto-limbic network that includes the anterior cingulate cortex, the medial frontal cortex, hippocampus and amygdala, a network connected in part via the white matter fibers of the cingulum bundle. One neural model posits that the symptoms of PTSD are the result of ineffective "top-down modulation" of the amygdala and limbic circuitry by the prefrontal cortex, in turn leading to impairment in emotion regulation. The abnormal MD that we detected in the cingulum bundle supports this model and may contribute to the disruption in neural networks that has been reported in functional imaging studies.

Clinical Relevance

Identification of distinct alterations in white matter connections in the PTSD brain should guide future research on the functional correlates of these tracts, and thus, provide a focus for possible interventions. Such alterations may also serve as biological markers for this disorder in the future.



The 2nd Annual TMII Symposium-2011



Figure 1. TBSS findings of right internal capsule/anterior thalamic radiation: decreases in MD. TFCE FWE corrected p < 0.10. Figure 2. DTI tractography of the cingulum bundle (green) and the anterior limb of the internal capsule (red).





Figure 3. DTI tractography quantified MD values of the anterior limb of the internal capsule and the cingulum bundle. A repeated measures MANOVA showed a between-subjects effect of PTSD on MD (F(1,18) = 6.429, p = 0.021).



Sensitization and Habituation of Neural Networks in Response to Aversive Social Cues in Borderline and Avoidant Personality Disorder Patients

Authors & Affiliations

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Introduction

Intense emotional reactivity is a hallmark feature of Borderline Personality Disorder (BPD), a highly prevalent, difficult to treat disorder with a suicide rate of 10%. The emotional reactivity of BPD is associated with many of the disorder's most maladaptive features such as suicidality, intense anger, unstable relationships, and identity disturbances. Avoidant Personality Disorder (AvPD), a distinct anxiety-related personality disorder, shares with BPD an excessive reactivity to social cues, but lacks the emotional instability characteristic of BPD. The neural bases of these emotional responses are poorly understood, but a failure to habituate or a sensitization to aversive social cues have been posited as underlying mechanisms.

Methods & Results

3.0 T BOLD fMRI images were obtained as patients with BPD (N=17), patients with AvPD (N=21), and healthy volunteers (HC's) (N=19) viewed, in a counterbalanced design, novel and repeated presentations of neutral and aversive pictures depicting social interactions. **Results:** When viewing repeated compared to novel aversive pictures, BPD patients showed greater activation in the right amygdala, fusiform gyrus, caudal anterior cingulate cortex (ACC), and left inferior frontal cortex, whereas HC's showed lesser activation. In contrast to BPD patients, AvPD patients showed greater activation to repeat vs. repeat pictures. In the hippocampus HC's showed a decreased activation to repeat vs novel pictures, while AvPD's showed little change.

Conclusion

These data suggest that in limbic, prefrontal and visual processing regions BPD patients sensitize to repeated presentations of negative social cues in contrast to HC's who habituate. Furthermore, in the caudal ACC, AvPD's, unlike BPD's, show greater activity when viewing novel compared to repeated stimuli and they do not show the habituation in the hippocampus seen in the HC's. The tendency to sensitize in the BPD's may account in part for the increased social reactivity of BPD patients in ongoing relationships. AvPD patients on the other hand, who are also sensitive to social situations, but are not emotionally unstable, do not show this sensitization, but show greater activity to novel stimuli.

Clinical Relevance

This study is the first to our knowledge to identify neural activity patterns which distinguish between two distinct personality disorders, characterized primarily by different types of social reactivity. By identifying the neural correlates of sensitization to repeated exposure to aversive stimuli in personality disorder patients, it could



crucially guide decisons about timing and tempo in shaping psychotherapeutic approaches to these patients.

Figures and tables

Borderline Patients vs. Healthy Controls Viewing Aversive Pictures

(Repeat - Novel) BPD > HC

Amygdala





Aging and Frontal-Temporal White Matter Integrity in Schizotypal Personality Disorder

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Introduction

Consistent with a schizophrenia-spectrum model, studies report gray and white matter abnormalities in the frontal and temporal lobes of individuals with schizotypal personality disorder (SPD) resembling those observed in schizophrenia. Fewer studies have used fractional anisotropy (FA), a diffusion tensor imaging (DTI) measure to examine the degree to which abnormalities in the white matter tracts connecting frontal-temporal regions observed in schizophrenia, are also seen in SPD. Aging effects on DTI in SPD have not been examined.

Methods & Results

DTI tractography was used to quantify the tract-specific FA of the anterior limb of the internal capsule (ALIC; frontal-striatal connections) and the inferior longitudinal fasciculus (ILF; temporal lobe connections) using quantitative-DTI tractography in 28 medication-naïve SPD patients and 32 age, gender, and education-matched healthy controls (HCs) (3T-Siemens-Allegra head-dedicated scanner). DTI tractography was performed using the Brute-Force Streamline Algorithm implemented in Matlab 2010b. Voxel-wise analysis was performed using the tract-based spatial statistics (TBSS) routines implemented in FSL to compare FA between groups on the white matter skeleton image. Tract-specific FA was entered into a Group (HC vs. SPD) x Age (Younger: 20-44 vs. Older: 45-60) x White-matter bundle (ALIC, ILF) x Hemisphere multivariate analysis of variance (MANOVA).

Compared with age-matched HCs, the younger SPD group had higher FA in the left hemisphere (averaged across white-matter tracts) and lower FA in the right hemisphere while in contrast, the older SPD group had lower FA in both hemispheres. Moreover, the HC-SPD differences were larger in the older than younger groups (Group x Age x Hemisphere interaction, F[1,56]=4.26, *p*=0.04, Wilks (Figure 1). Between-group comparisons were less robust when averaged across the young and old subgroups (Figure 2).

Conclusion

These findings suggest that white matter abnormalities in frontal-temporal tracts are more robustly observed in older compared with younger individuals with SPD. Aging is an



important factor in the integrity of both frontal-striatal and temporal lobe WM tracts in SPD. Clinical correlations will be presented.

Clinical Relevance

Abnormalities in frontal-striatal and frontal-temporal white matter connectivity among individuals with SPD provides important information about disconnectivity in schizophrenia-spectrum disorders. A better understanding of regional white matter abnormalities in schizophrenia-spectrum disorders may provide important psychopharmacological targets for the treatment of schizophrenia-spectrum disorders.

Figures

Figure 1. Fractional anisotropy (FA) averaged across the anterior limb of the internal capsule (ALIC) and the inferior longitudinal fasciculus (ILF) is shown for younger and older healthy controls and individuals with schizotypal personality disorder. Lower FA is observed in the older SPD patients, particularly the left hemisphere whereas, among the younger groups the differences are less marked.



Figure 2. Tractography analysis comparing the healthy control (n=32) and medication-naive SPD (n=28) groups (here the groups are averaged across young and old subgroups). Red area at top is the genu of the corpus callosum and red area at lower left is inferior longitudinal fasciculus. Both regions show a trend for lower fractional anisotropy (FA) in SPD patients compared with the healthy control group, p = 0.10, corrected.





CONFORMATION SENSITIVE PROBES IN PROTEIN MISFOLDING DISEASES

Authors & Affiliations

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Methods & Results

Luminescent Conjugated Polythiophenes (LCOs) and Luminescent Conjugated Oligothiophenes (LCOs) have during the resent years been a useful tool for the investigations of protein conformations in protein misfolding and amyloid formation. The gold standard for amyloid detection in tissues and cells as well as in protein solutions *in vitro* have been Congo Red and Thioflavine S or Thioflavin T. The LCP and LCO probes have been used for visualization of amyloid aggregates *in vitro*, *in situ* as well as *in vivo*. The LCPs and LCOs are conformation sensitive probes that emit different fluorescence spectrum, as a result of alternative protein binding. Different types of amyloidgenic proteins adopt different amyloid structures and gives raise to a unique spectral composition form the probes (Figure 1). Amyloid and pre-amyloid structures produced by the same protein do also display small spectral changes from the probes.

Conclusion & Clinical Relevance

By the use of LCPs and LCOs, the amyloid deposits in human post mortem tissue, as well as in transgenic mice are investigated. The probes will provide us with useful information about the protein misfolding pathways within human misfolding diseases.

Figures and tables



Figure 1. Spectral contribution for A β and tau aggregates found in *post mortem* human brain samples. Emission spectra of cerebral aggregates and spectral images from LCO stained human AD brain sections. Comparing spectra of human AD brain sections showing A β plaque (in blue) and Tau tangles (in red). Picture modified from Berg I et al 2010.



Optimized fMRI imaging protocol and custom-built olfactometer for studying the orbitofrontal cortex

Authors & Affiliations

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Introduction

Olfactory dysfunction has been associated with diseases as Alzheimer's disease, Parkinson's disease and obsessive-compulsive disorder (OCD). Problems such as the lack of affordable MR compatible olfactometers and susceptibility artifacts near the orbitofrontal cortex (OFC) make it difficult to perform imaging research using this modality. Here we present a solution using a cost effective custom-built olfactometer combined with an imaging protocol that minimizes the susceptibility artifacts in the OFC and olfactory cortex.

Methods & Results

The olfactometer (figure 1) is based on the principles of air dilution olfactometry and is composed of three main components – a controlled valves unit, a signal control unit and a Windows based laptop computer as well as an air compressor and a vacuum pump. The odorant control unit contains 12 solenoid valves to provide multiple odor stimuli. All connecting tubes are Teflon tubes (low absorbent material); except the tubing for the vacuum. An apparatus was built to determine the time-response characteristics of the olfactometer. Performance tests were conducted using different duration times of odorant delivery, a range of flow rates and tubing lengths. Nine normal healthy subjects participated – 5 males and 4 females, aged 20-43 years. Subjects were requested to count how many stimuli were given, pleasant/unpleasant, and identify what the stimuli were during the fMRI session.

Functional data was acquired in the coronal plane using a segmented EPI sequence. Because of the requirement for temporal resolution we were only able to cover 34 slices. An additional one time 68 slice set covering the whole brain was also acquired for co-registration and normalization purposes. Pleasant stimuli were chocolate, vanilla and banana; unpleasant were flatulence, cat urine and garbage. All were presented once for 10 seconds followed by a random jittered rest period of 30 to 42 seconds. Each scan was repeated 4 times with a pseudo randomized order of the odors. Imaging was performed using a Philips 3T Gemini MRI. Correlation maps were generated with FSL.

The characteristics of the olfactometer are shown in figure 2. The rise time of the olfactometer is approximately 1.3 seconds. The delay time due to delivery tube is linear with the length of the tubing (figure 3). Analysis of stimuli versus rest showed activations in the amygdala, insular cortex and DLPFC. In pleasant-unpleasant comparisons, activations were found in OFC and ACC, but not in the amygdala. Activations of one subject are shown in figure 4.

Conclusion

The custom-built, 12-channel, computer-controlled, MR compatible olfactometer was used to deliver the odorant stimuli to the subject in the MRI scanner. Subjects could identify the pleasantness and unpleasantness of the odorant stimuli, but could not completely identify what the stimuli were. There were significant increased activations not only in the OFC area, and were also found in the ACC when acquisition was in the coronal plane with segmented EPI-acquisition.

Clinical Relevance

With the custom-built MR compatible olfactometer and a workable protocol to study the OFC with fMRI paradigm, we can use these tools to further investigate obsessive-compulsive disorder.



Figures and tables





MRI detection of macrophages in rat focal ischemia using MPIOs

Authors & Affiliations

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Introduction

In stroke, significant brain damage is caused by immune cell infiltration. Anti-inflammatory drugs have been largely ineffective against stroke in clinical trials. Thus, noninvasive tracking of immune cells in stroke may facilitate a better understanding of the infiltration time line for effective treatment. MRI with micron sized iron oxide particles (MPIOs) has been shown to be a promising imaging modality for this purpose because of the capability for single particle detection. The purpose of this study was to investigate the feasibility of using MPIOs to monitor immune cell infiltration into focal cortical stroke.

Methods

Rats were infused with 400 pmol ET-1 into somatosensory cortex over an hour to produce a focal stroke. Laser Doppler flow was used to analyze cerebral blood flow reduction. Animals were immediately scanned at 11.7 T using a stroke MRI protocol consisting of ADC mapping, T2 mapping, and T2* weighted 3D gradient echo. Two hrs after the end of ET-1 infusion, animals were given i.v. injection of 1.63 μ m green fluorescent MPIOs (Bangs Laboratories) and scanned again at 1 hr, 24 hr, 48 hr, and 4 and 8 days later. Animals were sacrificed on day 8 and brains were perfused with 10% formalin and extracted for 50 μ m isotropic resolution 3D gradient echo imaging at 4.0 T. Sectioned brain slices were stained for microglia/macrophage markers IBA-1 and ED-1 and visualized with a confocal microscope.

Results

Ischemic tissue exhibited increase in T2 from 35 ms to 80 ms and decrease in ADC values from 7e-7 mm2/s to 3.5e-7 mm2/s, consistent with reports of ET-1 induced cortical stroke (2). T2* weighted 3D gradient echo images showed no signal voids in the stroke region at days 1 and 2. At day 4, dark contrast was present in the ischemic region, the volume of which increased at day 8. This accompanied reduction in both ADC and T2 insults. Histological analysis showed MPIO labeled cells positive for IBA-1 and ED-1, both microglia and macrophage markers, at day 8.

Conclusion

It is generally accepted that intense leukocyte infiltration of the brain parenchyma occurs 48-72 hours after stroke with peak monocyte and macrophage accumulation occurring 3 to 7 days after stroke. The principal finding in this study was that MPIO induced signal voids appeared in the ischemic region of the brain starting at 4 days post-stroke on T2* 3D GE images, consistent with the known peak accumulation of macrophages in the ischemic region. Whether these MPIOs are carried by circulating monocytes or are phagocytosed by resident microglia is difficult to answer due to the non-specificity of ED-1 for monocytes and microglia.

Clinical Relevance

The findings of this study are promising for using MRI-based cell tracking to specifically monitor immune cell infiltration in stroke at low cell numbers with MPIOs, which may facilitate more effective anti-inflammatory drug treatment. Furthermore, this approach to immune cell tracking can be easily



extended to other neurological diseases with strong immune responses such as Alzheimer's disease and multiple sclerosis.

Figures and tables



Top row: ADC maps of a representative slice at Day 0, 1, 2, 4, and 8 following stroke. **Second row:** T2 map from same slice. **Third and fourth rows:** Axial and coronal slices from 3D gradient echo MRI at the same slice. Red boxes illustrate MPIO induced dark signal voids, with expansion insets at Day 8. **IHC:** IBA-1 (red) and ED-1 (red) positive cells with nuclear DAPI (blue) staining. MPIOs are green.



fMRI identifies brain regions activated during the disruption of fear memories when using a novel behavioural reconsolidation interference procedure in humans.

Authors & Affiliations

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Introduction

After memory consolidation, memory was thought to be relatively resistant to change. However, recent findings demonstrate that memory is in fact prone to interference again, during a phase called reconsolidation, occurring each time we retrieve a memory. Non-invasive behavioural methods, such as extinction training, can be specifically timed to the memory reconsolidation phase in order to capitalize on this process for more permanent alteration of fear memory (Schiller et al., 2010). To identify the neural mechanisms underlying memory update through reconsolidation mechanisms, we used fMRI to examine the differences in brain activation when extinction occurred within or outside the reconsolidation window of fear memories.

Methods & Results

To condition fear, participants were presented with three colored squares on day one. Two colors (CS+A and CS+B) were paired with a mild electric shock and the third color (CS-) was never paired with the shock. On day two, to disrupt memory reconsolidation to one stimulus but not the other, only the CS+A was reminded without the shock 10 minutes before extinction training; after which all stimuli were extinguished using repeated presentations without the shock. On day three, the participants received fear reinstatement initiated by unsignaled shocks then followed by another round of extinction training and re-extinction to assess recovery of the fear memory. Consistent with previous studies, fMRI BOLD responses during acquisition produced increased amygdala activation to both CS+A and CS+B, but not CS-. On day two, as expected, extinction without the reminder (CS+B) deactivated the ventromedial prefrontal cortex (vmPFC). However, presenting the reminder 10min before extinction training failed to deactivate the vmPFC and decreased amygdala activation when compared to the extinction training of the CS+B. On day three, re-extinction assessed the recovery of the fear memory; there was less amygdala activation to the reminded stimulus (CS+A) compared to the non-reminded one (CS+B). Pilot data measuring skin conductance response, an indicator of fear, produced a trend indicating a reduction of fear during re-extinction in participants that received the reminder compared to participants that did not.

Conclusion

The activation pattern seen during extinction with and without the reminder suggests that the vmPFC is engaged differently when extinction training occurs during reconsolidation. The presentation of the reminder altered vmPFC activity during subsequent extinction training and led to decreased expression of fear as indicated by the amygdala and the skin conductance responses.

Clinical Relevance

These results may elucidate the underlying mechanisms for psychological treatments that rely on extinction training. These treatments are usually aimed to dissociate benign stimuli from maladaptive responses, such as drug-associated cues from craving in drug addiction and normal thoughts from excessive stress in anxiety disorders. This study can serve as a biomarker to predict the success of this novel form of extinction training, avoiding the use of potentially harmful pharmacological treatments.



Figures and tables











Aging impacts significantly on neuronal transport in normal mice but not in an accelerated mouse model of Amyloid Beta pathology.

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Introduction

Track-Tracing Manganese Enhanced MRI (TTMEMRI) is currently the only non invasive in vivo 4-D volumetric imaging technique to monitor neuronal transport perturbations. Using this approach, our group and others [1,2] confirmed the associated decrease in neuronal transport with both hallmark of Alzheimer pathology previously described [3]. In the present study, we sought to examine with the same approach an accelerated A β mouse model (Tg6799 5xFAD) expressing both APP and PS1 human mutations [4].

Methods & Results

Animals: Five 3-month-old and eight 6-month-old Tg 5XFAD transgenic model (Tg6799) [4] and seven 3-month-old and eight 6-month-old background matched wild type (WT) mice were used for this study. Imaging: A 7-T micro-MRI Bruker Biospec system was used. A 3D T1-SPGR sequence was utilized with: FOV = 19.2 x 19.2 x 9.6 mm, matrix= 128x128x64, isotropic spatial resolution = 150 μ m3, TR/TE = 15/4 ms, Flip angle 18°, 6 averages and an acquisition time of 15 min. Mice were imaged using a tract-tracing MEMRI (TT-MEMRI) protocol with 9 imaging time points, 1 pre and 8 post-intranasal injection of

MnCl2 (1.5 µL of a solution of 5M), were acquired subsequently at 1, 4, 8, 12, 24, 36, 48 hours and 7 days post injection. Data processing: All the MR datasets, were processed at the different timepoints, plotted and fitted to a previously described tract-tracing bolus model [5]. This enabled estimation of the following parameters: maximal signal (Smax), time to Smax (t2Smax), maximal upslope of the curves (Vmax= Δ Si/t) and the time to Vmax (t2Vmax). Time-curve plots obtained from wild type mice demonstrated visible difference over aging depicted in Fig.2. This unexpected decrease observed during normal aging was significant using both parameters Smax (p = 0.0016) and Vmax(p = 0.011) in the glomerular layer. In the mitral cell layer, in addition to the decrease in Smax (p = 0.0071) and Vmax (p = 0.0091), an increase in t2Smax (p = 0.0104) and t2Vmax (p = 0.0017) were also observed. Surprisingly Tg 5xFAD mice failed to show an age associated decrease in transport as evidenced the near superimposed time curves (Fig.3) suggesting maintained transport function. These data taken together demonstrated a significant difference in t2Vmax in the mitral cell layer (p = 0.035) at 6-month between WT and Tg suggesting either increased intake or transport. Fig.4 summarizes time to maximum slope in the mitral cell layer in both groups. Expression of A^β deposits was histologically confirmed both in the olfactory bulb and the piriform cortex in 6 month old Tg 5xFAD.

Conclusion

We show for the first time an early age associated decrease in neuronal transport and/or intake in (C57/B6xSJL) WT mice while the Tg 5XFAD appear to maintain their Mn transport profiles.

Our overall goal aims at better characterizing axonal transport impairment *in vivo* using a panel of different biomarkers over a long timeframe window [5].

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Clinical Relevance

In vivo MEMRI studies will allow longer term follow up enabling opportunities of multiple same subject trials and evaluations of new therapies for diseases affecting neuronal functional integrity.

Figures and tables





The Positive Role of the 'Negative System'

Authors & Affiliations

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Introduction

Fear learning is a rapid and persistent process that promotes defense against threats and reduces the need to relearn about danger. A central, yet neglected, aspect of fear modulation is the ability to flexibly shift fear responses from one stimulus to another if a once-fearful stimulus becomes safe or a once-safe stimulus becomes threatening. In these situations, the inhibition of fear and the development of fear reactions co-occur but are directed at different targets, requiring accurate responding under continuous stress. To date, research on fear modulation has focused mainly on the shift from fear to safety using paradigms such as extinction, resulting in a reduction of fear. The aim of the present study was to track the dynamic shifts from fear to safety and from safety to fear when these transitions occur simultaneously.

Methods & Results

We used functional neuroimaging in conjunction with a fear-conditioning reversal paradigm. During such a paradigm, one stimulus is paired with shock, and therefore associated with danger, while another stimulus is learned to be safe. Midway through the experiment, the stimulus outcomes are switched, and the subject must learn that the previously dangerous stimulus is safe, while the once safe stimulus now predicts electric shock. Fear was assessed using skin conductance response, a measure of autonomic arousal. Our results reveal a unique dissociation in the ventromedial prefrontal cortex (vmPFC) between a safe stimulus that previously predicted danger and a 'naïve' safe stimulus. The vmPFC is known to be involved in the inhibition of a fear response. It has also more recently been implicated as part of the 'default-mode system,' also known as the 'negative system because it shows reduced blood oxygenation level-dependent (BOLD) signal during externally directed tasks compared with a resting baseline. By looking at the network level, we found that not only the vmPFC, but the entire 'negative system' showed such responses to safe stimuli throughout the task. In contrast, another 'positive' system, including the amygdala and striatum, tracked the fear-predictive stimuli, flexibly flipping responses from one predictive stimulus to another.

Conclusion

The amygdala and the striatum have a prominent role in fear learning; they can flexibly adjust their responding during reversal and are positively correlated with fear-related arousal, but the ability to inhibit a prepotent fear response requires a network of additional brain regions. By adopting network approach to this data, we identify the broader circuitry involved in fear reversal, and provide strong evidence of the negative system's role in counteracting the learned contingencies of the 'positive system'. Dynamic fear responding depends upon the mirror activation of these two opponent brain networks. These results elucidate how fear is readjusted to appropriately track environmental changes, and the brain mechanisms underlying the flexible control of fear.

Clinical Relevance

The ability to flexibly readjust fear behavior when circumstances change is important for adaptive behavior. The failure to adjust to changing conditions may underlie some of the impairments observed in anxiety disorders.



Figures and tables

Average Skin Conductance Responses:



Positive versus Negative System Activations:





Regional Brain Metabolic Responses to Food Expectation Predict Food Preference

Authors & Affiliations

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Introduction

Brain glucose metabolism (BGluM) is a marker of brain function that can be used to map specific brain regions/circuits to behaviors. Using [¹⁸F]-fluoro-2-deoxyglucose (FDG) and small animal positron emission tomography (μ PET) we investigated regional changes in BGluM following the expectation of two conditioned food stimuli (bacon and chow) and related these changes to preference for each stimulus.

Methods & Results

Male adult outbred Sprague-Dawley rats were first exposed to a modified version of the conditioned place preference (CPP) paradigm, where each animal was conditioned for 30 min on alternate days and in contextually distinct chambers to expect either 5g of chow (4 days) or 5g of cooked bacon (4 days). Chow or bacon was provided 20 min into the conditioning session. Because of this delay, the cues associated with each food stimulus predicted expectation for either bacon or chow, as opposed to each stimulus *per se*. Rats were then tested for CPP, and on the following days, BGluM was assessed twice, once following exposure to the bacon-paired chamber, and once following exposure to the chow-paired chamber. Rats showed variability in CPP to food expectation, suggesting that at the individual-level, some rats preferred bacon, whereas others preferred chow, yet at the group-level, rats successfully paired the cues with stimulus delivery. Expectation to bacon activated several brain regions involved in reward, affective motivation, memory, motor planning and homeostasis and also increased the functional connectivity of these regions. Regression analysis showed that individual preference for bacon and chow-paired chambers was positively correlated with BGluM during expectation of each stimulus in regions associated with reward (nucleus accumbens), motivation/drive (caudate putamen, orbital cortex, hypothalamus), and arousal (thalamus).

Conclusion

Our findings illustrate the efficacy of an *in-vivo*, non-invasive and survivable strategy for mapping brain functional responses of expectation of two distinct foods to preference for each food at the individual level in rats. Using functional connectivity analysis we also show that brain circuitry implicated in craving and anticipation was activated during expectation of food and that different circutries were activated for each food stimulus. Using this type of imaging paradigm, one can screen rodents based on individual preference and in conjunction with genomics invesitgate susceptibility to food reward via the examination of individual genetic markers.

Clinical Relevance

This study shows that individual BGluM metabolism in craving/anticipation circuitry modeled in rodents was associated with individual preference for food. These results suggest that food preference may be influenced by individual differences in sensitivity of the food-reward circuitry to conditioned food stimuli. These individual differences may translate to individual vulnerabilities to compulsive eating and eventually obesity and addiction in humans.



Figures and tables



*Figure 1.*Statistical parametric maps showing increases (hot metal) and decreases (hot blue) in regional brain metabolism after bacon expectation.

Table 1. SPM: Regional Brain Metabolic Activations in Response to Chow or Bacon Expectation

Contrast	Puncorr	KE	t	ML	DV	ΑΡ	Region	Label
Bacon > Chow	0.03	13	2.3	1.1	-3.0	-3.9	CA1/DG	G
	0.03	19	2.2	-5.4	-4.7	1.2	S1ulp	C & D
Bacon < Chow	<0.001	97	6.6	0.6	-7.4	-9.8	DM Pons (PDT/Bar/Sph/LC)	J
	0.002		4.6	0.5	-7.4	-8.4	VTg	I
	0.001	9.0	5.5	-4.8	-8.6	-2.4	BLA	Е
	0.001	4	5.1	-0.4	-4.8	-11.8	CB lob. 4, 5	К
	0.002	12	4.7	-1.9	-3.5	3.8	M1	А, В, С
	0.002	15	4.3	1.9	-7.5	-14.2	SP5c	L
	0.003	6	4.3	0.1	-5.3	-3.4	DM. Thalamus (IMD/PVP)	F
	0.003	3	4.1	-2.4	-3.2	2.6	M1 (2)	А, В, С
	0.004	1	3.9	-2.9	-8.2	0.4	NAc	D

Table 1. Surviving clusters for each contrast (Bacon > Chow; Bacon < Chow) are shown along with their stereotaxic coordinates (Paxinos space: mediolateral (ML), dorsoventral (DV), antreroposterior (AP)), significance levels (Puncorr), effect (t) and cluster size (K_E) . CA1/DG (hippocampal CA1 region/dentate gyrus), S1ulp (upper lip region of somatosensory cortex), DM Pons (dorsomedial pons), PDT (posterodorsal tegmental nucleus), Bar (Barrington's nucleus), Sph (sphenoid nucleus), LC (locus coeruleus), VTg (Gudden's ventral tegmental nucleus), BLA (basolateral amygdala), CB lob. 4, 5 (cerebellum lobules 4 and 5), M1 (primary motor cortex), SP5c (spinal trigeminal nucleus caudalis), M. Thalamus (medial thalamus), IMD (intermediodorsal thalamic nucleus), PVP (posterior paraventricular thalamic nucleus), NAc (nucleus accumbens).







Figure 2. Graphic representation of the difference in functional connectivity observed between activated brain regions during expectation to chow (blue lines), expectation to bacon (black lines), and correlations among activated brain regions that significantly differed between the two conditions (red lines). Image was generated using IPA software (Ingenuity Systems, Redwood City, CA).


In vivo MRI of monkeys with white matter lesions: the relationship between structure and function

Authors & Affiliations

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Introduction

Recent advances have allowed us to acquire detailed imaging data from non-human primates *in vivo*. What value can such data provide when compared with the already high standard of images we can acquire from the human brain? One major contribution is that we can study the effect of targeted brain lesions in specific brain regions in the same subjects, an opportunity that rarely arises in human patients. We carried out a combined diffusion and resting-state MRI study in anesthetised rhesus monkeys to investigate the whole-brain effect of specific white matter damage.

Methods & Results

We acquired high-resolution structural, diffusion and resting-state images from anesthetized rhesus monkeys on two occasions: before and after a white matter brain lesion. Resting-state and anatomical scans were carried out in a horizontal 3T clinical scanner with a full bore. Monkeys were scanned in the sphinx position in an MRI-safe stereotaxic frame under isoflurane anaesthesia. They were intubated, ventilated and a range of physiological parameters were monitored. Whole-brain BOLD fMRI data was collected for 53 min and 26 s from each animal, using the following parameters: 36 axial slices; in-plane resolution, 2x2 mm; slice thickness, 2 mm; no slice gap; TR, 2000 ms; TE, 19 ms; 1600 volumes. A structural scan (three averages) was acquired for each macaque in the same session, using a T1-weighted sequence (voxel size 0.5x0.5x0.5mm).

We analyzed the resting-state and diffusion data to assess whether: (i) structural changes in the white matter can be detected not just close to the lesion but also distally; (ii) changs in functional networks reflect these structural changes. The diffusion data were corrected for distortion and motion artefacts. The functional data were preprocessed with motion correction and removal of respiratory artefacts. Analysis was carried out using the FSL package (www.fmrib.ox.ac.uk/fsl). Alterations in white matter fractional anisotropy were observed not only close to the lesion site but also distally. We were able to visualise resting-state correlations in the anaesthetised monkey brain and compare these with the white matter alterations.

Conclusion

Evidence from human studies suggests that structural alterations on a macroscopic level underlie functional changes even in the adult brain. We investigated whether this could also be true in the case of specific white matter brain damage. In addition to revealing in more detail the relationship between structure and function, these findings can also inform us about the basis of resting-state correlations.

Clinical Relevance

This study demonstrates that we can study changes in the non-human primate brain following a lesions in a way which provides more specificity than in human patients. We will be able to apply this technique to study the effects of brain damage in the same subjects before and after the lesion,



in several animals, to allow us to study how reproducible these effects are. This may help to develop diagnostic tests for neurobiological disorders which result in white matter changes.

Figures and tables



Figure 1 – 1x1x1mm resolution diffusion data from a rhesus macaque monkey acquired on a 3T scanner using a custom-made 4-channel radiofrequency coil. Left – several acquisitions can be averaged to produce high levels of signal throughout the brain. Right – color-coded data showing the principal diffusion directions: left –right (red), dorsal-ventral (blue) and anterior-posterior (green).



Figure 2 – Resting-state BOLD data from a group of healthy monkeys superimposed on an example structural scan. One component of an independent components analysis reveals the default mode network with activation in regions including the ventromedial prefrontal cortex, posterior cingulate cortex and hippocampal formation.



White Matter Microstructure in Adults with Attention-Deficit/Hyperactivity Disorder

Authors & Affiliations

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Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is an early-emerging neurodevelopmental disorder, characterized by age-inappropriate levels of inattention and/or hyperactivity/impulsivity, that commonly persist, with varying levels of severity, into adulthood. Neuroimaging studies suggest functional and structural abnormalities in a number of brain structures including the prefrontal cortex and cingulate cortex. However, few studies have tested the relationship between microstructural characteristics of the white matter traversing these regions symptom persistence among adults diagnosed with ADHD in childhood.

Methods & Results

A sample of twenty-three adults diagnosed with ADHD in childhood and 24 well-matched controls, all of whom participated in a longitudinal study of ADHD, were included in the analyses. We utilized diffusion tensor imaging (DTI) to test for differences between these groups in fractional anisotropy (FA) several regions of interest, including the frontal white matter, the anterior and posterior cingulum bundle, and the genu, body, and splenium of the corpus callosum. Higher FA values are thought to reflect greater directional coherence of diffusion in white matter, thereby indicating greater directional coherence and axonal integrity. The ROIs were chosen due to their roles in attention and inhibitory control circuitry. We performed two sets of preliminary analyses to: (1) test for differences in FA between adults diagnosed with ADHD in childhood and controls; and (2) examine the relationship between FA and scores on a standardized self-report measure of ADHD symptoms among the ADHD group. Contrary to previous reports, there were no group differences in FA in the frontal white matter or cingulum bundle. A significant group difference emerged in the body of the corpus callosum (p = .03) with the ADHD group demonstrating significantly less FA than the control group. Correlation analyses revealed significant (p<.05) negative relationships between hyperactive/impulsive symptoms and FA in right anterior cingulum bundle (r = -.43), the genu of the corpus callosum (r = -.43), and a significant (p < .05) negative relationship between inattentive symptoms and FA in the splenium of the corpus callosum (r = -.45).

Conclusion

The results of these preliminary analyses suggest that increased FA in the cingulum bundle, and the genu and splenium of the corpus callosum might be associated with decreased ADHD symptoms in adulthood. However, it is possible that the varied symptom presentation in the adult ADHD group occluded differences between those with childhood ADHD and controls. Therefore, further study, with a larger sample size is needed to determine whether there are differences in FA between controls, those with childhood ADHD who demonstrate symptom persistence, and/or those who demonstrate symptom remittance.

Clinical Relevance

Identification of white matter circuits that demonstrate less axonal integrity among those with ADHD compared to controls might help elucidate findings from other structural and functional imaging methods, providing a clearer view of the systems involved in the neurodevelopment of the disorder.



Functional connectivity of thalamus during response preparation among young adults with ADHD

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Introduction

Children and adults with Attention-Deficit/Hyperactivity Disorder exhibit difficulty eliciting and maintaining levels of arousal and response preparation for optimal task performance. Functional connectivity of the thalamo-cortical network may play a role in arousal/response preparation deficits in ADHD. Therefore, we utilized regression-based analysis of psychophysiological Interactions (PPI) to test for differences in thalamo-cortical functional connectivity in adults with childhood ADHD.

Methods & Results

Twenty adults who were diagnosed with ADHD in childhood and 20 controls (mean age = 24.2, SD = 1.5) were scanned using event-related fMRI during an cued reaction time (RT) task. Brain activity related to response preparation is modeled by subtracting neural responses to non-cue events from neural responses to Cue events. Functional neuroimaging results from the full sample were used to identify an appropriate seed region for the connectivity analysis. PPI is a regression-based method of functional connectivity that tests for differences in the regression slope of activation between brain regions due to the differential response to the signal from one region (seed) under the influence of different experimental contexts (e.g., cue vs. non-cue). Both groups demonstrated significant peaks of activation in the dorsomedial (DM) nucleus of the thalamus (controls: x = -8, y = -18, z = 8; Probands: x = -6, y = -16, z = 2; p < .001 uncorrected, k = 100). The peak of activation for each group was used as the seed for individuals within the respective groups. 15 controls and 15 probands who exhibited activation within DM thalamus, as confirmed by the Talairach Daemon software, were included in the PPI analysis. The significance level for the PPI was set at p < .05, k = 100. Probands and controls demonstrated cue-evoked activity of similar regions associated with response preparation (i.e., thalamus, putamen, parietal cortex, mid-cingulate, supplementary motor area), with decreased intensity among probands within cingulate, SMA, and parietal cortex. The PPI analysis revealed group differences in the pattern of thalamic connectivity. Compared to probands, controls demonstrated greater functional connectivity between thalamus and 'task positive' anterior cortical regions involved in response preparation (e.g., BA 8 and DLPFC), and temporal regions associated with target identification. Compared to controls, probands demonstrated greater cue-related functional connectivity with subcortical structures (caudate/putamen) and the precuneus, which is part of the default mode network.

Conclusion

Group differences in functional connectivity between thalamus and cortical regions suggest that young adults who were diagnosed with ADHD in childhood demonstrate altered connectivity between thalamus and other task-positive regions responsible for response preparation, as well as with regions of the default network. Thus, there is evidence for disruption in the interplay between task-positive and default mode regions among young adults with a history of ADHD diagnosis.

Clinical Relevance

Individuals with ADHD might demonstrate subtle differences in neural activation and connectivity that might inform the development of appropriate treatments for the disorder.





Figure 1. Although controls and probands demonstrated similar patterns of activation, they demonstrated dissimilar patterns of connectivity.



Figure 2. Controls demonstrated greater connectivity with 'task positive' regions, while probands demonstrated greater short-range connectivity with caudate/putamen and the precuneus, which is part of the default mode network.



Effect of Gender on Fractional Anisotropy Measures in Traumatic Brain Injury

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Introduction

Traumatic brain injury (TBI) may produce long-lasting neurological and cognitive effects. Previous brain imaging studies using diffusion tensor imaging have found reduced fractional anisotropy (FA) and diffuse axonal injury (DAI) in TBI patients. In the current study, we used diffusion tensor imaging to investigate the effects of TBI on white matter and correlated these findings with a calculated injury severity score and data from neuropsychological assessments in males and females.

Methods & Results

Twelve subjects diagnosed with mild to moderate TBI and eleven age and gender-matched controls were scanned once after completing a battery of neuropsychological testing. Images were obtained on a Philips Achieva 3T X-Series Whole-body MRI. A diffusion-weighted image (TR=5682.4 ms, TE=70.0 ms, FOV=21.0 cm, matrix = 104x106, 38 slices, thickness = 2.5 mm, no skip, 32 gradient directions, max b=1200 s/mm²) was acquired using a head-dedicated SENSE 8-channel head coil for each subject. Images were eddy-current-corrected and fractional anisotropy (FA) maps were calculated using FSL (www.fmrib.ox.ac.uk/fsl). In-house software developed in Matlab 2009 was used for ROI data collection. The corpus callosum was traced on a midsagittal slice. Tracing was performed on an edge enhanced FA image using a Sobel filter. The average FA of the CC was calculated. Whole-brain analysis of FA was done separately using TBSS in FSL. Prior to scanning, participants were assessed using the Brain Injury Screening Questionnaire (BISQ), the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Brief Health Questionnaire (BPHQ), Cognitive Failures Test (CFQ) and severity of TBI. The BISQ is a self-report of cognitive, physical, and emotional symptoms, the BDI and BPHQ are indicators of depression, the BAI an index of anxiety, and the CFQ is a self-report measure of functional cognitive failures experienced in daily life. Injury severity was classified using a 7-point scale ranging from 1 (No loss of consciousness, no confusion (i.e., no TBI) to 7 (Loss of consciousness greater than 4 weeks in duration). T-tests were used to determine group differences and correlations between FA and neuropsychological measures were computed.

Conclusion

The neuroprotective effects of female sex hormones may have buffer damage to white matter in female subjects and may account for the decreased FA in white matter observed in male TBI subjects but not in female TBI subjects. These effects may have also permitted a broader spectrum of damage which may have contributed to the correlation of severity and FA observed in the female TBI group.

Clinical Relevance

Our findings indicate gender may be a factor in the neurological damage following a traumatic brain injury. Further elucidating this difference may point to potential treatment approaches for traumatic brain injury patients.





Figure 1. Results of the whole brain FA analysis using TBSS. Row A depicts areas where male TBI group displayed significantly lower FA compared to male controls. Row B indicates there were no significant differences between female TBI and female control groups.



Figure 2. Results of the whole brain FA (A) and corpus collasum FA (B) analysis for TBI and control subjects separated by gender. Female controls (n=5) did not significantly differ from female TBI (n=7) group in either analysis technique. Male TBI (n=5) had significantly reduced FA compared to male controls (n=6) in whole brain and CC analysis techniques.

Neuropsych	Mean Control	Mean	t valua	đ		N Controla	<u>N</u> mTDI
Assessment	Control	midl	t-value	<u>a1</u>	<u>p</u>	<u>N Controls</u>	midi
BDI	1.60	12.67	-3.42	20	0.0027	10	12
BAI	1.64	6.5	-2.69	21	0.0137	11	12
BPHQ	12.00	16.33	-2.65	21	0.0150	11	12
CFQ	17.82	45.75	-4.28	21	0.0003	11	12
Severity							
Measure	1	2-7					

Table 1. Neuropsychological assessment measures for controls and TBI subjects.



Evaluating Neural Effects of Social Skills Treatment for Children with Autism Spectrum Disorders

Authors & Affiliations

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Introduction

Despite the fact that social skills impairments are the most persistent and pervasive symptoms affecting individuals with autism spectrum disorder (ASD), treatments targeting social skills have been the subject of few controlled investigations. The available literature suggests that cognitive behavioral techniques (CBT) are used commonly and may be efficacious for improving social functioning. While several neuroimaging studies have found that individuals with ASD underactivate key brain regions involved in social cognition, we have shown that activity in normative neural networks can be increased significantly by providing children with ASD with explicit instructions to attend to important social cues. This suggests that a CBT approach to social skills treatment may increase not only social responsiveness at the behavioral level, but also the activation of normative neural systems that support social functioning. This study aims to examine the neural effects of a CBT approach to social skills (in comparison with a play-based control group) using two previously validated tasks that probe targeted skill domains: 1) interpreting communicative intent, and 2) processing gaze direction in emotional faces.

Methods & Results

Thirty-one verbally fluent children with ASD, 8-11 years of age, were randomized to the CBT or social play comparison group. Both treatment approaches consisted of 12 weekly 90-minute sessions (4-6 children in each group) with a concurrent parent group. Twenty-one children (17 boys, 4 girls) had successful pre- and post-treatment fMRI scans.

While undergoing fMRI, children viewed drawings of characters in a conversational setting while listening to short scenarios ending with a comment made by one of the characters. Participants decided whether the speaker meant what she said. Following treatment, children in the CBT group showed greater activity in the medial prefrontal cortex (MPFC), the ventrolateral PFC, and the superior temporal gyrus (STG)/temporal pole relative to baseline (Fig. 1). In contrast, children in the social play comparison group did not show any regions of increased activity post- vs. pre-treatment. When directly comparing the two groups on changes in brain activity, we found that children in the CBT group showed greater increases in regions relevant for theory of mind (MPFC), emotion recognition (STG/temporal pole) and emotion regulation (ventrolateral PFC) compared to children in the play group (Fig. 2). Interestingly, improvement in social behavior (measured by the social relations subscale of the Children's Communication Checklist) was associated with increased activity in the MPFC, the left STG/temporal pole, and the amygdala bilaterally across groups (Fig. 3).

Conclusions and Clinical Relevance

While these data are preliminary, it is encouraging that the CBT group showed increased activity in regions playing a key role in social cognition after a relatively brief intervention period. By exploring the relationship between changes in brain activity and changes in social behavior, we can begin to develop hypotheses about the neural mechanisms underlying response to treatment. If these results hold, they suggest that a cognitive behavioral approach may have some efficacy at the neural level.





(Fig. 2) CBT > Play: Post-treatment – Baseline



(Fig. 3) Correlations with the Children's Communication Checklist





Cross-sectional and Longitudinal Reproducibility of Rhesus Macaque Brain Metabolites: Proton MR Spectroscopy at 3 T

Authors & Affiliations

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Introduction Due to similarities in physiology, anatomy and cellular function to its human counterpart, the rhesus macaque brain is used as a "pre-clinical" model system for various diseases, such as multiple sclerosis (1), and neuroAIDS (2). Because of the prohibitive cost of performing destructive studies, especially if they involve serial observations, non-invasive imaging methods, such as MRI for morphology and proton MR spectroscopy (¹H-MRS) for metabolism, are often the modalities of choice. Indeed, *in vivo* ¹H-MRS can monitor the *N*-acetylaspartate (NAA), creatine (Cr), choline (Cho) and *myo*-inositol (*m*I) signals, the putative markers for neuronal integrity, cellular energy status, membrane turnover rates and glial proliferation, respectively (3). Although the reproducibility metric is essential for adequately powered study design and to distinguish real changes from biological and instrumental noise (4), its global average values for this species and their range have not been reported. Our two primary goals, consequently, are: (*i*) to establish, using test-retest 3D multivoxel ¹H-MRS, the macaque brain's average NAA, Cr, Cho and *m*I concentrations and their cross-sectional (*inter*-animal) and longitudinal (*intra*-animal) variations in a large, 28 cm³ (~35%) volume of interest (VOI) in the macaque brain at 3 T; and (*ii*) demonstrate the method's sensitivity to distinguish pathological change from biological and instrumental variations.

Methods & Results All experiments were done in a 3-T MR imager (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) with a circularly-polarized transmit-receive human knee coil. To guide placement of the ¹H-MRS VOI, sagittal and axial turbo spin echo MRIs (TE/TR=16/7430 ms, 140×140 mm² field-of-view (FOV), 512×512 matrix, 2.0 mm sagittal and 1.2 mm axial slice thickness) were acquired. A 4.0 cm anterior-posterior (AP) × 3.5 cm left-right (LR) × 2.0 cm inferiorsuperior (IS) = 28 cm^{3} ¹H-MRSI VOI was then centered on the corpus callosum. The VOI was excited using PRESS (TE/TR=33/1440 ms) with two 2nd-order Hadamard encoded slabs (4 slices) interleaved within every TR. These slices' planes were encoded with 16×16 2D-CSI over an 8×8 cm² (LR×AP) FOV to yield 224 voxels, (0.5 cm)³ each, in the VOI. The 224 VOI spectra were each frequency-aligned and zero-order phased in reference to the NAA peak, then summed, retaining individual spectra linewidth and improved SNR by $224^{\frac{1}{2}} \approx 15$. Relative levels of the *i*th (NAA, Cr, Cho, *m*I) metabolite in the *j*th animal were estimated from their peak areas, S_{ij} , using parametric spectral modeling and least-squares optimization software by Soher *et al.* (5). The S_{ij} were then scaled into absolute concentrations relative to signals from a 2 L sphere of 12.5, 10.0, 3.0 and 7.5 mM NAA, Cr, Cho and mI in water, by phantom replacement as described previously (6). Five healthy adult (3 females, 2 males; all 3 years old; 4.3 – 5.6 kg weight) rhesus macaques were scanned one to three times. Each was tranquilized as described previously (7). One macaque was subsequently infected by intravenous injection with simian immunodeficiency virus (SIV) and then rescanned 6 weeks later. Animals were under constant veterinary supervision. To determine *intra*-animal reproducibility, two macaques each underwent three separate ¹H-MRS sessions and one macaque underwent two. To determine inter-animal reproducibility, two more macaques each underwent one session (total of 10 sessions from 5 animals). To determine the variance due to VOI repositioning, four back-to-back scans were acquired at each session with all experimental parameters kept unchanged for a total of 40 full 16×16×4 3D ¹H-MRS data sets.

Results: Mean NAA, Cr, Cho and *m*I concentrations in the macaque brain were: 7.7 ± 0.5 , 7.0 ± 0.5 , 1.2 ± 0.1 and 4.0 ± 0.6 mM/g wet weight (mean±standard deviation). Their *inter*-animal coefficients of variation (CV) were 4%, 4%, 6% and 15%; and the *intra*-animal CVs were lower still: 4%, 5%, 5% and 4%, much better than the 22%, 33%, 36% and 45% *intra*-voxel CVs.

Conclusion As shown in Fig. 1, summing all phased and aligned elements in the VOI achieves $\times 4 - \times 11$ fold better CVs than a single-voxel method by exploiting **B**₀ homogeneity (narrow linewidth) across individual 0.125 cm³ voxels. The $\langle 15\% inter$ -animal CVs indicate good cross sectional similarity between healthy animals. The longitudinal *intra*-animal reproducibility is even better, exhibiting CV's below 10% across the board. These CVs can be used to compute power tables geared to detect (and identify as "real") predetermined metabolic changes that are due to either disease progression or treatment response.

Clinical Relevance Both *inter*- and *intra*-animal results are encouraging given past reports of NAA changes larger than 25% within lesioned areas after ischemic stroke (7), and in frontal cortical GM after acute simian immunodeficiency virus (SIV)-infection (8). This is demonstrated by the subtle (but diffuse) changes brought about by SIV infection in a macaque brain 6 weeks post infection (Fig. 2); changes are both clearly visible and significant with this approach in a single animal.

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Fig. 1. Box plots displaying the 25th, median, 75th (box) and \pm 95%-tiles (whiskers) of the NAA, Cr, Cho and *m*I concentrations distributions for all voxels of all scans (*intra*-voxel), sessions across all macaques (*inter*-animal), sessions of an animal (*intra*-animal) and all scans for a session (*intra*- session). Note the ×4 to ×11 fold, improvement in both *inter*- and *intra*-animal reproducibility of the sums versus with the single voxels. Insert: Box plots of the GM, WM and tissue fraction (GM_f, WM_f, and T_f) distributions in the VOIs of all animals. Note the narrow distribution of tissue types, indicating minimal GM/WM/CSF partial volume repositioning error in the proposed approach.





Cognition Emotion Integration in Anterior Insular Cortex

Authors & Affiliations

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Introduction

Both cognitive and affective processes require mental resources. However, it remains unclear whether these two types of processes work in parallel or in an integrated fashion. A widely held view, *functional specialization* assumes that mental faculties are instantiated by distinct brain areas. However, functional localization is likely an oversimplification that should be augmented with the principle of *functional integration*, which bears greater ecological validity, especially in the case of higher-level cognitive and emotional processes. The anterior insula (AI) and the anterior cingulate cortex (ACC) have been considered as potential candidates for such integrative processing due to their neuroanatomical complexity and their functional roles in many psychological processes.

Methods & Results

In this functional magnetic resonance imaging (fMRI) study, we investigated the functional interaction of these two processes using an empathy for other's pain paradigm, with simultaneous manipulation of task demand and stimulus valence. Eighteen healthy human adult participants viewed color photographs showing other people's hands and feet in painful or neutral situations while performing tasks of low (body-part judgment) and high cognitive demand (laterality judgment). Participants also performed an explicit pain judgment task that served as a functional localizer of empathetic pain. Behavioral data showed increased reaction times and error rates for painful stimuli compared to non-painful stimuli under laterality judgment relative to body-part judgment, indicating a behavioral interaction between task demand and stimulus valence. Both regions of interest and whole brain analyses showed activity in bilateral anterior insula (AI) and somatosensory cortex (SI), but not posterior insula, for main effects of task demand and stimulus valence. Importantly, task demand and stimulus valence showed a significant interaction in AI, SI and regions of the frontoparietal network.

Conclusion

The current study provides the first empirical evidence to show that AI is a key node in a brain network that serves as the anatomical basis for cognition-emotion integration. We speculate that functional integration of cognition and emotion is adaptively advantageous and parsimonious. Such integration does not exclude the distinction between cognition and emotion, but rather, suggests that the difference might be phenomenological instead of ontological.

Clinical Relevance With further exploration of the integrative, dynamic nature of cognition and emotion, we may acquire a better understanding of neurobehavioral function and as a result, of its deficits in psychiatric illnesses.



Figure 1. Sample stimuli of the experimental stimuli set of 144 digital color photographs. There were eighteen photographs in each of the eight permutations of three variables with two conditions each (pain: pain, no pain; laterality: left, right; and body part: hand, foot). Participants were asked to choose between "hand" and "foot" for body-part judgment (TB), "left" and "right" for laterality judgment (TL), and "no pain" and "pain" for pain judgment (TP).



Figure 2. fMRI ROI results. (*A*) ROI analysis of the parameter estimates of anterior insula (AI) and anterior cingulate cortex (ACC) for four experimental conditions (TB-no pain, TB-pain, TL-no pain, TL-pain) derived from the task localizer (TP vs. baseline). (*B*) ROI analysis of the parameter estimates of posterior insula (PI) and primary somatosensory cortex (SI) for four experimental conditions derived from the empathy localizer (TP-pain vs. TP-no pain). TB: task body-part. TL: task laterality. TP: task pain. Error bars represent standard error of the mean (S.E.M).





Automated Modeling of Targeted Non-Invasive Electrical Stimulation of the Brain with Multiple Electrodes

Authors & Affiliations

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Introduction

Conventional transcranial direct current stimulation (tDCS) applies weak constant currents to the surface of the scalp. High-density tCDS aims to improve targeting by using a number of gel-based electrodes instead of the conventional saline-soaked large-pad sponges. To achieve maximal performance in clinical applications, it is important to apply an appropriate current to the region of interest accurately through correctly placed electrodes. The goal of this study is to develop a fully automated targeting software for high-density tDCS. This software can establish a head model for a given subject, compute the optimal electrode configuration and simulate the current flows through each electrode and the brain.

Methods & Results

Structural magnetic resonance imaging (MRI) data of the brain was used as the basis for an anatomical model of tissue conductivities. The MRI (T1-weighted) was segmented into six tissue types with different electrical conductances: gray matter, white matter, cerebrospinal fluid (CSF), bone, soft tissue (skin) and air. The segmentation was performed using a probabilistic segmentation routine ("newsegment") which runs on Statistical Parametric Mapping 8 (SPM8). An improved Tissue Probability Map (TPM) with 6 tissue types covering head and neck was developed at the Center for Advanced Brain Imaging and was used in the segmentation. After SPM8 automated segmentation the resulting six segments were then edited, both manually and automatically to correct segmentation errors, such as "floating" and "empty" voxels, discontinuities in the CSF, and other corrections necessary to insure accurate current flow modeling. Manual "clean-up" was performed using ScanIP, which required days to weeks of user interaction and familiarity with anatomical MRI. Automated "clean-up" was based on morphological criteria such as continuity of CSF, connectivity, adjacency of tissues, minimal structure sizes, etc. These were implemented using morphological operations in Matlab. Then, again using Matlab, 89 electrodes and gels were automatically placed on the head following the conventions of the standard 10-10 international system (nasion, inion, pre-auricular points are provided by the user). Four additional electrodes are placed on the neck to provide distant references. After this, the six segments, plus placed electrodes and gels, were imported into ScanFE to generate a tetrahedral mesh with each segment defining an area of uniform conductivity. This mesh was then imported into Abaqus to solve for the current distribution in the head using finite element model (FEM) techniques for a specific pair of electrodes (Fp1 and Iz were chosen). The final current distributions computed with the FEM were compared between manual and automated segmentations. The whole process is shown in Fig. 1, and the results are shown in Fig. 2 – Fig. 6.

Conclusion

The automated method can achieve comparable performances in the "clean-up" of segments and the final current simulation. This means that the manual correction can be replaced by automated algorithm, thus the whole process can be automated. Then the software user can select any pair of electrodes to see the current distribution between them when doing tDCS therapy.

Clinical Relevance

tDCS is currently used as a therapy for certain brain disorders such as depression and stroke. This software can be used as a potential tool for doctors to guide the tDCS therapy for optimal focality and intensity in a subject-specific brain area.





Fig.5 (Left) The full 10% 74-channel arrangement from Easy Cap; (Middle, Right) Results of electrode placement on the skins from manual and automated methods, respectively



Fig.6 The simulated current distributions from stimulating electrode (Fp1, above left eye) to reference electrode (Iz, at the inion). From left: currents around Fp1 in gray and white matter from manual and automated methods; currents around Iz from manual and automated methods



Diffusion Weighted Imaging Facilitates Detection of Spinal Multiple Myeloma and Assists in Diagnosing Equivocal Lesions

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Introduction

Traditional multiple myeloma spine surveys employ a combination of unenhanced T1, STIR and T2 weighted images. Some clinical imaging schemes employ contrast enhanced T1 techniques. Each technique and sometimes all can be equivocal in lesion detection, particularly in elderly or debilitated patients with heterogeneous bone marrow composition/signal. Diffusion weighted imaging (DWI) can highlight the presence of lesions by sensitizing to the restricted motion of water. The utility of this sequence in the spine has been studied extensively with regards to characterizing vertebral body fractures (benign versus pathologic) and vertebral body lesions (metastatic versus benign) with mixed results. We attempt to document the incremental contribution and clinical utility of DWI in improving the visual conspicuity of lesions and refining the characterization of equivocal osseous lesions in patients with multiple myeloma.

Methods & Results

METHODS

We retrospectively reviewed spine surveys from 15 pathology proven MM patients performed from January 2010 - December 2010 [1-19 lesions per patient (total=105)]. 7 patients (45 lesions) received contrast. Routine sequences included sagittal T1, STIR and T2 weighted images. When feasible, CE sequences were performed. Sagittal echo-planar DWI sequences were added (3-6 directions, B value 500-800).

Initially, T1, STIR and (if available) CE sequences were consensus reviewed twice by 2 experienced neuroradiologists. Diagnostic confidence for each lesion was graded 1-4 (1=benign; 2=equivocal; 3=probable; 4=definite). Lesions were regraded after DWI supplementation. Lesion conspicuity differences were recorded as "more," "less," or "same."

A non-normal data distribution mandated use of nonparametric methods including Kruskall-Wallis, Wilcoxon Signed Rank and Sign tests (α =0.05). Bowker's test for symmetry and Kappa coefficient estimations evaluated the significance and magnitude of diagnostic confidence score differences for routine and DWI techniques.

RESULTS

Diagnostic confidence was greater with DWI (μ =3.4) than without (μ =3.0) [p=0.0029], was upgraded in 35%, identical in 49% and downgraded in 16% of lesions. 11 lesions (10%) were missed. 1 lesion (1%) was visible only on DWI. On DWI, 51 lesions (49%) were more conspicuous, 32 (30%) equally conspicuous, and 22 (21%) less conspicuous.

Conclusion

Fast and easy to perform, DWI improves MM lesion detection and characterization, especially in problematic locations.

Clinical Relevance

- Diffusion imaging of the spine is a fast sequence (3 minutes)
- Diffusion imaging assists in detecting multiple myeloma lesions
- Diffusion imaging assists in diagnostic confidence that a questionable lesion is myeloma





Confidence in Diagnosis

		ROU	TINE		
DWI	BENIGN	EQUIVOCAL	PROBABLE	DEFINITE	TOTAL
BENIGN	0	9	4	0	13
EQUIVOCAL	0	3	2	1	6
PROBABLE	2	8	5	1	26
DEFINITE		15	11	43	70
TOTAL	3	35	22	45	105

DWI Effects on Imaging Interpretation

- 43/105 lesions (41%) deemed definite (4) with both techniques
- 11/105 lesions (10%) upgraded from probable to definite
- 15/105 lesions (14%) upgraded from equivocal to definite
- 9/105 lesions (9%) downgraded from equivocal to benign
- 1 Lesion (1%) Not Visible on Routine Images; Only DWI Sequence



Dual Energy Spectral CT of the Instrumented Spine: Tuned Monochromatic Imaging Improves Quality Over Traditional Techniques

Authors & Affiliations

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Introduction

Analysis of the spectral Hounsfield signal behavior of dual energy spectral CT (DESCT) scans obtained in the presence of spine instrumentation provides the opportunity for selection of the appropriate energy level to minimize artifact and optimize image quality. In this study, we sought to determine the energy level that would maximize signal-to-noise ratios in the spinal canal of instrumented spines and to compare these tuned monochromatic (MC) images to those produced from routine MC (70 keV \approx 140 kVp polychromatic) images.

Methods & Results

METHODS

Twenty-five patients who previously had undergone spinal intervention were imaged on a GE CT750HD machine using DESCT. The data were processed and spectral Hounsfield unit curves were generated based on a region of interest placed in the spinal canal at the level of the spinal hardware. Optimal keV levels for signal-to-noise ratios (SNRs) were identified in each case and sets of multiplanar tuned MC images were generated at those same levels. These tuned images were compared with standard 70 keV MC images and evaluated for artifact around the hardware, artifact obscuring the spinal canal, and overall diagnostic quality.

RESULTS

Evaluation of spectral Hounsfield unit curves demonstrates that optimal SNRs were obtained at a 110 keV energy level. Comparison of tuned MC images to the standard MC 70 keV images demonstrates significant reduction in noise and artifact in the spinal canal. Furthermore, the optimal images for evaluation of the spinal canal were adequate for the assessment of other aspects of the study (e.g., degenerative disease).

Conclusion

The optimal energy level for imaging the instrumented spine is 110 keV. Monochromatic images tuned to 110 keV produced images with less artifact in the spinal canal and better hardware visualization, with superior overall quality.

Clinical Relevance

Tuned MC imaging's ability to optimally visualize the osseous and nonosseous tissues surrounding the hardware of instrumented spines enables evaluation for suspected colocalized disease (e.g., hardware loosening, recurrent or persistent radiculopathy or tumor, cement leakage, etc) otherwise often hidden from view when relying upon traditional polychromatic techniques. As a result, we now perform all instrumented spine studies using dual energy spectral CT tuned to 110 keV.









The 2nd Annual TMII Symposium





Abstracts, Nanomedicine category





Silk-silica fusion proteins for bioengineering applications

Authors & Affiliations

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Introduction

Incorporation of genetic engineering tools in biomaterial design allows for fine tuning of the material properties with respect to the application site *in vivo*. Mimicking nature gives a wide range of biodegradable materials to be tested. Spider dragline silk is the strongest known fiber that rivals synthetic kevlar, famous from its great tensile strength. Also, biosilica of diatoms is synthesized under physiological conditions with nanoscale precision of diatom cell wall patterns. Our goal was to combine the genes responsible for spider dragline silk strength and controlled silica mineralization to construct a novel biomimetic material to be tested in bone remodeling studies.

Methods & Results

Recombinant DNA techniques were used to design protein fusions and tailor nanocomposite material features for tunable performance. The impact of modifications at the molecular level as well as different chemistries of silica mineralization were assessed for their influence on material properties such as morphology, structure, and mechanics. Outcomes were aevaluated by interactions of human mesenchymal stem cells (hMSC) with the bioengineered nanocomposites towards osteogenesis. Genetic control over silk-silica fusion protein designs coupled with materials processing resulted in remarkable control of silica morphology and distribution leading to 3D porous silica networks, clustered silica nanoparticles (SNPs), or single/isolated SNPs.

Conclusion

Preliminary studies with hMSCs show support of silk/silica films towards cell attachment and upregulation of osteogenic gene markers, the latter pronounced highly for porous silica network.

Clinical Relevance

We anticipate that these novel biomaterials have widespread applications in tissue regeneration and drug delivery due to the ability to regulate the precise location and features of the silica. The silk component serves as an organic scaffold that controls material stability and allows multiple modes of processing. The silica component, due to the tight control of feature sizes and distributions serves as an important osteoinductive component in biomaterials with potential control of remodelling rate and tissue regeneration outcomes *in vivo*.

Figures and tables



Figure 1. SEM images of silica morphologies on the surface of silk films mineralized at different protein concentrations: a) 5% wt/vol, b) 2.5% wt/vol, and c) 2.5% wt/vol with addition of glycerol.



A Surface Activatable Nanoparticle System for Targeted Therapy

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Introduction

Nanotherapies applied to cancer may have significant benefits in terms of drug efficacy as well as reduction of the adverse effects usually associated with classical treatments. Moreover the conjugation of polymers and target-specific molecules to the nanoparticle surface may improve nanoparticle pharmacokinetics and tumor targeting. The purpose of this project is to develop a surface activatable nanoparticle platform for the targeting of specific tissue like $\alpha v \beta_3$ -expressing breast cancer cells via RGD-peptides, while minimizing the exposure of the targeting ligand RGD to $\alpha_{V}\beta_{3}$ -expressing cells in the circulation (Fig. 1A). To achieve this goal it will be necessary to shield the RGD-peptides present on the nanoparticle surface by introducing cleavable-long PEG chain lipids in the corona. Once the nanoparticles accumulate in the tumor interstitial space, by the enhanced permeability and retention (EPR) effect, the long PEG-chains will be selectively cleaved by matrix metalloproteinase-2 (MMP-2) highly expressed by the tumor. This process will enable the RGD-peptide to be available for targeting. To test the feasibility of this system, a biotin-avidin model was used. Important challenges of the study are: to vary the PEGylation degree of the lipid layer while maintaining the nanoparticle morphology, and to find the best coating composition that enables an efficient ligand shielding.

Methods & Results

Experiments were performed to validate the ligand shielding property of the particle platform. Oil-in-water nanoemulsions coated by a mixture of long PEG chain phospholipids (PEG 3000-DSPE), short PEG chain phospholipids (PEG 550-DSPE), cholesterol and biotin-short PEG chain phospholipids (biotin-PEG 300-DSPE) as targeting moieties, were synthesized. All the components were dissolved in chloroform, mixed in the exact ratio (Fig 1B) and a lipid film was obtained using a rotary evaporator. Twenty mg of soybean oil were stabilized with 7.1 µmole of mixed PEGylated phospholipids, 1.42 µmole of cholesterol and 0.0142 µmole of Rhodamine-lipid. After the film hydration, the crude emulsion was sized using a microfluidizer. Even modifying the ratio between the coating components, all the formulations had a similar size after synthesis, around 75-90 nm in diameter. To evaluate the biotin-avidin interaction two approaches were used. Nanoparticle aggregation was induced by incubating the formulations (3 mM) with 6 µM of avidin at room temperature for 30 min, 2 hours and 4 hours. Subsequently sizes were measured by dynamic light scattering (DLS). As shown in Figure 1C, the exposure to avidin increased dramatically the relative size of "cleaved" samples. The aggregation was more pronounced in those samples that contained high amount of biotin-PEG 300-DSPE. Just a partial size increase was observed in the "shielded" samples. The interaction avidin-biotin was also quantified measuring the nanoparticle binding on avidin-coated wells. Fig. 1D clearly shows a low binding of the "shielded" samples compared to the "cleaved" samples.

Conclusion

These preliminary data clearly demonstrate the effect of different amounts of biotin-PEG 300-DSPE as well as of PEG 3000-DSPE on the nanoparticle aggregation and on the ligand shielding respectively. The avidin-biotin system is an easy model that helps us to assess the functionality of basic nanoparticle platform, before we apply and test the formulations containing MMP-2-cleavable PEG lipid *in vitro*.





Figure 1. (A) Proof of concept. (B) Mole percentages of the coating components in the formulations. (C) Nanoparticle aggregation after avidin exposure. (D) Nanoparticle binding on avidin coated wells.



Theranostic iron oxide nanoparticles as morpholino delivery systems

Authors & Affiliations

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Introduction

Low-density lipoprotein (LDL) enables the transport of serum cholesterol; however, high LDL levels put patients at risk for arteriosclerosis. The PCSK9 protein is responsible for the destruction of internalized LDL receptors (LDLr) from cell surfaces. As an anti-arteriosclerosis therapy, we are developing a multifunctional nanoparticle (NP) system to image and deliver morpholino (MO) sequences with the aim to silence PCSK9 gene expression, thereby increasing LDL receptor levels and decreasing LDL levels.

Methods & Results

Methods: Nanoparticles (A) were synthesized in a one-step process by mixing oleic acid coated iron oxide cores, amphiphilic polymer poly (Maleic Anhydride-Alt-1-Octadecene) substituted with 3-(Dimethylamino) propylamine, biocompatible polymer polyethylene glycol (PEG), carboxy PEG, and fluorophore-NIR-664-labelled lipid in chloroform:methanol mixture before dripping into boiling water. Because hepatocytes express asialoglycoprotein receptors that bind to galactose-terminated glycoproteins, we attached galactosamine in a NHS-EDC reaction to the nanoparticle with the aim of improving hepatocyte uptake. The nanoparticles were characterized for core size and morphology via transmission electron microscopy (TEM), and size distribution via dynamic light scattering (DLS). In vitro HepG2 uptake of galactose-targeted NP (Gal-NP) and control NP (PEG-NP) was analyzed by fluorescence imaging. Six MO sequences were designed to block the pre mRNA splicing of human PCSK9 gene. These MOs were screened for their effect on LDLr in HepG2 cells via Western blotting techniques. In addition, to develop a high-throughput assay for nanoparticle-MO delivery efficacy, two MOs that target the luciferase gene in SC002 cells were designed and luciferase expression was assayed by luminescence studies. To enable MO incorporation into the nanoparticle, we synthesized 1,2-dimyristoyl-snglycero-3-phosphoethanolamine (DMPE) liposomes whose amine groups have been modified to react with thiol-appended MO via dithiol linkage. The reaction was monitored by UV spectroscopy.

Results: TEM showed that the iron cores were individually coated with the polymer-lipid mixture (B). DLS showed that nanoparticle's effective diameter ranged from 28 to 38 nm. HepG2 cells uptake of Gal-NP was not greater than PEG-NP, which suggested that galactose attachment may not have occurred. Western blots showed LDLr upregulation (C) in MO-treated HepG2 cells. Also, MOs were demonstrated to silence luciferase genes with three days being the optimal incubation time (D). Amines on DMPE were successfully modified as seen by characteristic absorbance of reaction by-product at 343 nm (E.a). However, DMPE attachement to thiol-appended MO did not occur as seen by lack of absorbance at 260nm characteristic of MO (E.b).

Conclusion

The nanoparticle coating process is effective in vitro. MOs silence genes in both HepG2 and SC002 cells. Galactose and MO attachment to the nanoparticles needs to be optimized, before evaluation of in vitro gene knockdown.

Clinical Relevance

With further development, this nanoparticle delivery platform could potentially be employed as an anti-arteriosclerosis therapy in patients.





amine modification reaction on DMPE. b. DMPE after MO attachment.



Liposomal Formulation of Chelating, Cell-Impermeant Dyes for Ratiometric Fluorescent Imaging of Mn(II) Ions

Authors & Affiliations

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Introduction

Manganese(II) ion is of interest in biology and medicine for several reasons, including its use in Mn(II)-enhanced magnetic resonance imaging. Our earlier work (Gruppi, Liang, Royzen, Bartelle, Turnbull, and Canary, submitted) established that acetoxymethyl esters of calcein blue (CB) and fluozin-1 (FZ) could be delivered to cells and used for two-color imaging of Mn(II) ion. However, the two dyes could not be delivered in precisely equal amounts, diminishing the advantage of a ratiometric assay since calibration would be required. Here, we seek to deliver the dyes in precisely equal amounts using a liposomal formulation.

Methods & Results

A variation of (Hirnle et. al 1991) was used to prepare liposomes. Briefly, 32 mg POPC and cholesterol were mixed in chloroform in a molar ratio of 3:1 (POPC: Cholesterol), and dried under nitrogen. For aqueous solutions of dye, CB was readily and completely dissolved, whereas FZ would not dissolve without the addition of NaOH. Three formulations of dye: FCLS—Fluozin Green:Calcein Blue in a 1:1 molar ratio (0.83mg FG, 0.57mg CB); CLS—Calcein Blue (1.4 mg); FLS—Fluozin-1 (1.4 mg), in1mL water were added to lipids, incubated at 50C for 45 mins., sonicated until the mixture became clear and opalescent, then washed by to remove unincorporated dye. Dynamic Light Scattering revealed the smallest particle size for CB liposomes, the largest for FZ, and an intermediate size for mixed dyes (Table 1). Fluorescence was read against the linear range of aqueous standards of known dye concentration of FZ and CB. Subsequently, 20% ETOH was then added to half the wells to lyse the liposomes and release the dye contents—DLS showed a nearly total dissolution of liposomes, and an increase in small molecule content (Table 1). Changes in dye fluorescence upon lysis were greater for FZ than for CB (Fig. 2). The concentration of FG was less in intact liposomes (Fig.3).

Conclusion

We show that the problem of maintaining the stoichiometric ratio of a hydrophobic and a hydrophilic dye in aqueous medium can be solved by incorporation of the dyes into a liposomal delivery vehicle. The dyes will reversibly partition to the microenvironment of least free energy, while incorporated, without loss of material upon lysis. The increased radius of curvature of FZ containing liposomes, and dramatic increase in fluorescence upon liposomal lysis suggests that the hydrophobic nature of FZ causes it to intercalate with the liposomal bilayer, making the bilayer less flexible, and partially quenching the FZ emission. CB, which is mostly contained within the hydrophilic core, does not exhibit these phenomena.

Clinical Relevance

The modification of solubility or cell permeability of active imaging agents of cell metabolism is usually undesirable, and sometimes impossible, as the characteristic chemical property that defines the agent may also be altered in an unpredictable way. The self-assembling properties of the liposome solve this problem in much the same way that native lipoproteins solve similar solubility issues, such as cholesterol transport, in vivo.



The 2nd Annual TMII Symposium

Table 1) DLS results for liposome

formulations before and after lysis. Samples were analyzed by DLS (Wyatt Technology) at 25C using Dynamics [®] software. Following lysis, virtually all macromolecular structures have disappeared. Results for lysed FG liposomes were similar (not shown).

Formulation	R (nm)	%Pd	MW (kD)	%Mass
FGCBLs	23.4	64.2	5390	100
CBLs	17.4	6.6	2696	93.8
	68.1	17.8	65482	6.2
FGLs	33.4	22.1	12336	99.8
	197.5	28.6	791199	< 1
After Lysis in 20% ETOH				
FGCBLs	0.2	11.9	<1	100
	15.3	11.9	1978	<1
	80	14.2	95457	<1
CBLs	1.7	10.9	11	95.2
	16.3	24.8	2318	4.5

Figure 1) Bright field (column 1) and fluorescence microscopy (40X) of HEK 293 cells treated with 22.6 μ M CB and 3.6 μ M FZ. A) In the absence of Mn²⁺ there is normal signal from CB (column 2) but negligible signal from FZ, (column 3). B) with the addition of 5mM Mn²⁺.



Figure 2) Increase of normalized fluorescence for intact liposomes in water (blue line) and upon lysis of liposomes in 20% ETOH (red line). A) Results for the mixed dye formulation shows the 455nm peak emission corresponding to CB, and the 530nm peak for FG for the single particle. B) Results for the CB only particle, at 455nm, and the FG only particle at 530. Error bars show high and low values for n = 2.

Figure 3) Apparent concentration of dye as calculated against the fluorescence of known standards. Standards emission was linear within the range of sample concentration. Evidence of fluorophore quenching is seen in the fact that the FG emission in the dual-dye formulation appears to be significantly lower than CB. Upon lysis it can be seen that the molarity of the dyes is equal.







Gold nanoclusters as a targeted contrast agent for computed tomography imaging of angiogenesis

Authors & Affiliations

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Introduction

Computed tomography (CT) imaging is one of the best techniques for imaging atherosclerotic plaque in the coronary arteries. Atherosclerosis is a progressive inflammatory disease of the arteries, characterized by a build up of lipids and inflammatory cells. This tissue build up stimulates the development of new blood vessels to supply oxygen and nutrients. The formation of this neovasculature (angiogenesis) is an important marker for plaque development. Hence CT contrast agents to characterize angiogenesis would be valuable to study atherosclerosis. To address this need, we have developed $\alpha_{v}\beta_{3}$ -integrin (a marker for angiogenesis) targeted gold nanoclusters (A), characterized them and studied their targeting to angiogenic cells with fluorescence and CT imaging.

Methods & Results

Dodecanthiol coated gold nanocores were synthesized via the Brust method. These cores were dissolved together with distearoyl phosphoethanolamine (DPSC), DSPC-polyethyleneglycol (PEG), a rhodamine lipid and maleimide-DSPC-PEG in 9:1 chloroform:methanol. This mixture was added dropwise to hot, stirred deionized water. The resulting lipid coated nanoparticles were then appended with either the RGD or the RAD peptide (the RGD peptide is specific for the $\alpha_{v}\beta_{3}$ -integrin whereas the RAD peptide is a mismatch control sequence). The mixture was then centrifuged on a 1.25 g/ml KBr gradient to isolate gold nanoclusters. RGD targeted nanoclusters were termed RGD-NC, while RAD nanoclusters were termed RAD-NC. Transmission electron microscopy confirmed the formation of nanoclusters of gold cores (B). Further characterization included dynamic light scattering and CT phantom imaging.

The targeting of the nanoparticles was evaluated in human umbilical vein endothelial cells (HUVEC), a cell line well known to overexpress the $\alpha_{\nu}\beta_{3}$ -integrin. First, nanoparticle uptake was evaluated with confocal microscopy (C, D), where preferential uptake of RGD-NC by the HUVEC was observed. Furthermore, HUVEC were incubated with either RGD-NC, RAD-NC (both 0.5 Au mg/ml) or media only. After 30 minutes, the cells were washed, harvested and pelleted. CT images of the pellets were acquired with a 256-slice clinical scanner at 140 keV, revealing significant CT contrast in the cells incubated with RGD-NC, whereas less contrast was observed in the cells incubated with RAD-NC, indicating specificity of the RGD-NC for the angiogenic HUVEC cells (E).

Conclusion

We have successfully produced high payload gold nanoclusters that act as CT contrast agents. In vitro experiments indicate that these nanoclusters target angiogenic cells, as indicated by both CT and fluorescence imaging. The next steps will be to perform experiments in animals to determine their in vivo targeting/contrast properties.

Clinical Relevance

These nanoparticles represent a potential route to imaging angiogenesis in atherosclerotic plaque using CT imaging.



Legend: A) Schematic depiction of $\alpha_{v}\beta_{3}$ integrin-targeted gold nanoclusters. B) TEM image of the nanoclusters. C) and D) Confocal microscopy images of HUVEC cells incubated with rhodamine labeled nanoclusters. E) CT image of HUVEC cell pellets incubated as labeled.



Gold labeled low density lipoprotein nanoparticles: a novel platform for imaging lipoprotein biointeractions

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Introduction

Low density lipoprotein (LDL) plays a critical role in cholesterol transport to peripheral tissues, which is crucial for the maintenance of cell membranes. However, intra-arterial LDL deposition is one of the main causes of atherosclerotic plaque formation and progression that can ultimately lead to clinical events such as myocardial infarction and stroke. To facilitate study of these highly important natural nanoparticles, we developed novel technology to allow labeling of native human LDL with gold nanocrystals and rhodamine lipids (Au-LDL). This enables studying LDL interactions with hepatocytes and macrophages using a variety of imaging techniques, including computed tomography (CT), transmission electron microscopy (TEM) and fluorescence techniques.

Methods & Results

LDL was isolated from human plasma by sequential ultracentrifugation (Methods Enzymol. (1986) 126;6). Lipid coated gold nanocores incorporating rhodaminewere synthesized as described previously by Cormode *et al.*(Nano Lett (2008) 3715-3723). Native LDL particles were sonicated in water for 5 min with the gold cores in a 1 mg ApoB100 to 2.8 mg gold ratio (Fig.1A). Empty LDL and Au aggregates were removed via centrifugation on a dual KBr density gradient. Negative stain TEM confirmed that the gold nanocores had entered the hydrophobic triglyceride/cholesterol ester center of LDL, and showed a similar size and morphology to native LDL (Fig. 1B). The diameters of Au-LDL, determined by dynamic light scattering (DLS) and TEM, were 21.3±3.2nm compared to 23.6±2.9nm of native LDL, indicating little difference between Au-LDL and native LDL. Further characterization was done using both denaturing and non-denaturing gel electrophoresis. We found that this method was vastly more effective for labeling LDL with gold nanocores than the classic Krieger method (Methods Enzymol. (1986) 128;608-613).

In vitro incubations and competition assays were performed in hepatocytes (HepG2) and murine macrophages (J77A4.1). CT scans of the cell pellets, as well as a visually detectable color difference of the pellet incubated with Au-LDL alone, the competition assay, and the control showed that the cells take up Au-LDL in a saturable, receptor-like process (Fig.1C). Fluorescence microscopy performed on HepG2 cells, showed uptake of Au-LDL, a decreased uptake in the competition assay, and no uptake in the control (Fig. 1D).TEM was performed on the cell pellets and confirmed that the gold nanocores were taken up by the cells (Fig. 1E).Future experiments will involve CT imaging and microscopy studies in atherosclerotic mice.

Conclusion

Native human LDL particles loaded with gold nanocrystals have a comparable composition and morpohology, and behave similarly to unlabeled LDL particles. HepG2 cells take up Au-LDL in a receptor-like manner, as detected by CT, confocal microscopy and TEM.

Therefore Au-LDL can likely be used as a surrogate marker to study LDL interactions and atherosclerotic plaque formation.

Clinical Relevance

Au-LDL has potential to be used for studying and imaging the behavior of LDL in atherosclerosis and other diseases. This could be an important new step in better understanding the onset and progress of atherosclerosis and the lipid metabolism in human.







Formulation, Development, and Characterization of Iron-encapsulated Polymer Nanoparticles

Authors & Affiliations

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Introduction

This project involves a multidisciplinary approach to treatments for heart disease and atherosclerosis, utilizing various imaging techniques and nanotechnology-based tools. This abstract focuses on the development of a theranostic nanoparticle for effective plaque targeting and delivery of anti-atherosclerotic drugs. The particle platform includes core contrast agents for imaging, a polymer core for controlled release of drug, and a high drug payload (see Fig. 1). The work presented here is a summary of the development of the first two aspects of this platform: the incorporation of iron oxide as an MRI contrast agent into a polymer core nanoparticle.

Methods & Results

Poly(lactic-co-glycolic acid) (PLGA) with 50:50 monomer ratio was used with 10nm and 18nm oleic acid-coated iron crystals obtained from NN Labs. Soybean lecithin and DSPE-PEG 2000 were used in a 7:3 molar ratio as a lipid coating. Initially, a method developed by the Farokhzad Lab was used for the formulation of monodisperse PLGA particles of a controllable size. However, this method was not compatible with any of the solvents needed to effectively dissolve the iron oxide crystals. As an alternative method, a concentrated solution of lipid micelles was made via a controlled chloroform evaporation method, in which a solution of lipids in chloroform was slowly added to warm water and stirred. A second slow addition of a PLGA/iron oxide solution in dichloromethane was then added to the micelles, sonicated, and stirred at room temperature. Extent and timing of sonication were optimized, as well as solvent systems and lipid ratio. While this increased the level of iron encapsulated within the particles, the percent entrapment was still quite low. Seemingly, mixing iron oxides and PLGA is unfavorable. This is unsurprising when considering the predominantly aliphatic oleic acid used as a capping ligand on most commercially available iron oxide nanocrystals, in contrast to the polyester composition of PLGA. Using a synthetic acid-terminated short poly(lactic acid), we attempted a capping ligand transfer with the oleic acid coated iron. This dramatically increased the encapsulation of iron within the particles, however IR spectra were not consistent with the assumption that extensive ligand exchange had taken place.

Conclusion

While we have managed to achieve significant loading of iron oxides into PLGA nanoparticles, we will attempt to improve loading efficiency by increasing the compatibility of the iron crystals and the polymer core. Future work will focus on a more efficient ligand exchange on existing crystals, as well as the synthesis of new iron crystals using more compatible capping ligands.

Clinical Relevance

The particle design incorporates a PEG coating for biostability, a polymer core for extended drug release, and superparamagnetic iron oxides for MRI contrast. It is part of a larger project funded by the NIH to develop a theranostic drug delivery tool for the treatment of atherosclerosis.



Figure 1. **Nanoparticle Design.** The formulated nanoparticle has several different functionalities. The core is made up of iron oxide nanocrystals and statins embedded in PLGA polymer. The purpose of the polymer core is controlled release of drug, while the iron allows for visualization by MRI. The phospholipid surface incorporates PEG chains for increased stability and controlled curvature.



Figure 2. Effect of Ligand Exchange on Iron Inclusion. The TEM images above show two particles prepared using an identical procedure, one with oleic acid-coated iron oxides (A) and one with iron oxides having undergone a ligand exchange with a low molecular weight PLA (B).



A novel reconstituted high-density lipoprotein based nanotherapy for atherosclerosis

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Introduction: Inflammation drives progression and destabilization of atherosclerotic plaques. Statins constitute the backbone for strategies to lower cardiovascular risk because of their potent cholesterol lowering capability. Whereas preclinical studies have shown that statins also have anti-inflammatory effects, the clinical relevance is hampered by the limited bioavailability of orally administered statins. To enhance the anti-inflammatory effects we developed statin-loaded reconstituted high-density lipoprotein nanoparticle ([s]-rHDL). The advantages of [s]-rHDL comprise its long half-life in plasma and the targeting to macrophages in atherosclerotic plaques.

Methods & Results: To focus on anti-inflammatory effects, we used ApoE KO mice, whose cholesterol level is unaffected by statins. First, to evaluate macrophage targeting by [s]-rHDL. Gd-DTPA labeled [s]-rHDL was administered intravenously to ApoE KO mice (n=3). In vivo T1-weighted MR imaging (9.4 T Bruker MRI scanner) revealed strong signal enhancement in the abdominal aortic wall (Fig1 a-d). Moreover, accumulation of [s]-rHDL was observed in the aortic valve and branching areas in the mice administered with Cy5.5 labeled [s]-rHDL by NIRF imaging (Fig1 e, f) and specific uptake of [s]-rHDL by macrophages was revealed by fluorescence microscopy (Fig1 g-I). Furthermore, flow cytometry confirmed that macrophages robustly took up rHDL in plagues, and the more differentiated macrophages took up more rHDL than less differentiated macrophages (Fig1 m-r). Second, to assess the anti-inflammatory effects of [s]-rHDL, mice (n=62) were put on a high fat diet from 4 weeks of age onwards. At 14 weeks after diet initiation, mice were randomized to receive either placebo (n=15), oral simvastatin (10 mg/kg per day; n=15), intravenous rHDL (10 mg/kg ApoAI twice per week; n=16), or intravenous [s]-rHDL (15 mg/kg simvastatin with 10 mg/kg ApoAI twice a week; n=16) for 12 weeks. In vivo MR imaging of abdominal aorta was performed in 8 mice of each group at baseline, 6, and 12 weeks after randomization. Progression of vessel wall thickness was significantly inhibited in [s]-rHDL-treated animals compared to oral simvastatin, rHDL, and placebo groups (Fig2 a). To objectively and guantitatively analyze histological sections (n≈4000), we built an automated Matlab procedure. Histology results at termination showed that plaque size (hematoxylin phloxine saffron staining) in the [s]-rHDL treated group was significantly reduced compared to rHDL and placebo groups (Fig2 b). Importantly, the macrophage positive area (anti-CD68 immunostaining) in the [s]-rHDL treated group was profoundly reduced compared to oral simvastatin, rHDL, and placebo groups (Fig2 c).

Conclusion: [s]-rHDL successfully delivers simvastatin to macrophages in atherosclerotic plaques as revealed by in vivo MRI imaging, ex vivo imaging, and histology. As a consequence, the [s]-rHDL formulation improves the anti-inflammatory effects of statins, which can be expected to improve its atheroprotective effects compared to oral statin therapy.

Clinical relevance: Currently, the recurrence rate in the acute phase post coronary events is considerably high, which drives the development of new therapies. By targeting inflammation in vulnerable atherosclerotic plaques, [s]-rHDL nanotherapy may help patients pass the high risk vulnerable period following acute coronary syndrome.




Figure 1. Targeting of [s]-rHDL to macrophages in atherosclerotic plaques. For in vivo imaging, ApoE KO mice with advanced plagues were subjected to MR imaging before after and 24 hours intravenous administration of Gd-DTPA labeled [s]-rHDL. Strong signal enhancement was found in aortic wall (a-d). For ex vivo imaging, 24 hours after administration of Cv5.5 labeled [s]-rHDL ([Cy5.5-s]-rHDL) or saline (control), aortas were imaged with near infrared fluorescence imaging (NIRF). Specific accumulation of [Cy5.5-s]-rHDL in aortic valve and branching areas was observed (e,f). Subsequently, cross-sections were made from aortic valve areas (indicated by the white arrow in 'e') and imaged with

fluorescence microscope (g, i, k). The sections were stained with macrophage marker CD68 (h, j, l). Macrophage specific uptake of nanoparticles was observed (k, l). For flow cytometry, mice were intravenously administered with either [Cy5.5]-rHDL or rHDL. Cells in aortic walls were collected and macrophages were identified by proper markers. Macrophages were further identified as Gr1^{hi} and Gr1^{lo} populations, and fluorescence was detected with flow cytometry (BD Bioscience, LSRII) (m-q). Quantitative mean fluorescence intensity increase fold was calculated with FlowJo software (r).



Figure 2. Efficacy of 12 weeks low dose infusion of [s]-rHDL on inhibiting the progression of atherosclerosis. MRI scans of abdominal aorta three time at

points on 32 mice (8 per group), and normalized wall index showed that [s]-rHDL infusion halted progression of wall thickening more efficiently than other treatments (statin: simvastatin treatment, placebo: saline infusion, rHDL: rHDL infusion) (a). Efficacy of 12 weeks infusion of [s]-rHDL was assesed by plaque size (hematoxylin phloxine saffron stain), and [s]-rHDL infusion group had small plaque size compared to other groups (b). The macrophage positive area (anti-CD68 stain) reduction in [s]-rHDL treated group was more dramatic than other groups (c).



In vivo MRI-based cell tracking using Bio-MPIOs

Authors & Affiliations

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Introduction

PLGA-encapsulated iron oxide nano- (NPs) and microparticles (MPs) are important advancements for cellular MRI as they incorporate large amounts of iron and possess favorable degradation characteristics. Furthermore, PLGA is an FDA-approved material with a long history of use in drug delivery, enabling a clear trajectory towards potential clinical use. Here we describe design improvements to PLGA-encapsulated iron oxide NPs and MPs, cell differentiation and cytokine release experiments, and ultimately, the first *in vivo* demonstration of MRI-based cell tracking using PLGA-encapsulated iron oxide MPs.

Methods & Results

<u>Magnetic particles:</u> PLGA encapsulated magnetic NPs and MPs were fabricated with increasing amounts of magnetite and assayed for iron content and size.

<u>Cell studies</u>: Magnetic PLGA NP and MP cell labeling kinetics were assayed over 24 hrs in culture using dose and time dependent experiments. Labeled mouse mesenchymal stem cells (mMSCs) were differentiated down adipogenic and osteogenic lineages. Adult-derived rat neural progenitor cells (NPCs) labeled with PLGA NPs or MPs were cultured for 2 weeks in differentiation media. Cells were then fixed and stained for Tuj1 (immature neurons), GFAP (astrocytes), and O4 (oligodendrocytes). Lastly, macrophages labeled with magnetic PLGA particles were assayed for cytokine release.

<u>In vivo MRI</u>: MRI-based cell tracking was tested using a paradigm of *in vivo* labeling and tracking of endogenous rat NPCs. PLGA MPs (20 μ l of 10 mg/ml particles) were injected into the lateral ventricles of the brains of 6-week old rats (n=4 for each particle type) and rats underwent high resolution (100 microns isotropic) 3D gradient echo MRI at 11.7T over the course of two weeks. NPCs phagocytose these particles at the ventricle and carry them as they migrate to the olfactory bulb, revealing dark contrast on T₂*-weighted MRI.

<u>RESULTS:</u> Maximal particle loading was achieved using a feed ratio of 2:1 magnetite to PLGA, both for NPs and MPs, incorporating as much as 84 wt% magnetite. SEM images of these particles are shown in Figure I. Magnetic cell labeling with both NPs and MPs occurred in a time and dose dependent manner with 92-95% viability (Supp. Fig. I). Equivalent osteogenic and adipogenic potential (Supp. Fig. II) was observed for mMSCs labeled with all particle types. Immunocytochemistry on labeled NPCs (Fig. III) showed that the cells retained their ability to differentiate into neurons, astrocytes, and oligodendrocytes. Macrophages labeled with all particle types secreted high levels of TNF-alpha and IL-6 following stimulation with LPS (Supp. Fig. III). Magnetically labeled NPC migration into the olfactory bulb was observed via MRI over 2 weeks (Fig. II).

Conclusion

Magnetic PLGA MPs enabled the visualization of the NPC migration pathway with as good, if not better fidelity than the Bangs MPIOs traditionally used for this. This is the first in vivo demonstration of MRI-based cell tracking using Bio-MPIOs and is encouraging for the development of these particles for clinical use.

Clinical Relevance

Bio-MPIOs can be used to label and monitor the behavior of transplanted cells via MRI. Unlike the previous MPIO (Bangs) proposed for this purpose, Bio-MPIOs are biodegradable and have a much clearer trajectory for clinical translation.





Figure: I. SEM of NPs and MPs (top) with physical and relaxometric characteristics (bottom). II. MRI-based cell tracking of endogenous neural progenitor cell migration using Bio-MPIOs. Magnetically labeled cells are detected as dark contrast moving from left to right during 14 days of MRI. Prussian blue staining for iron indicates contrast is from Bio-MPIOs. III. Immunocytochemistry of neural progenitor cells labeled with green fluorescent particles showing expression of multiple differentiation markers (red)

Supplementary Figure





Figure: L Cell labeling kinetics of NPs and MPs. For a) 2:1 MP using three doess in Table 2 (dotted line is Dose 1, dashed line is Dose 2, solid line is Dose 1) and b) for all four particles using only Dose 2 (dotted line is 1:1 NP, short dashed line is 1:1 MP, long dashed line is 2:1 MP, and solid line is 3:1 MP. Nalues are means $+7 \times 5EM$, n=4. IL. Optical microscopy of adiopenesis (a-g) and osteogenesis (i-p) for labeled cells (as indicated) and unlabeled cells (d,l). h) labeled cells prior to differentiation. p) magnified image of o. III. ELISA of TNF- α (black bars) and IL-6 (gray bars) release following labeling for 16 hours. $\sqrt{3}$ is stimulated with LPS, - is unstimulated. Also shown are labeled cells as indicated.



Magnetic cellulose particles as relaxation switches for environmentally sensitive cellular MRI

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Introduction

Magnetic relaxation switches have been developed using iron oxide particles that cluster in the presence of a stimulus, but the potential of using such systems to monitor intracellular events *in vivo* via MRI has not been investigated. Here we demonstrate the fabrication of magnetic cellulose particles that are relaxometrically sensitive to digestion by cellulase. In the 'off' state, the particle remains intact. When switched 'on' through the cleavage action of cellulase, the iron oxide cores disperse, changing the relaxivity of the agent.

Methods & Results

<u>Particles</u>: 10-nm magnetite nanocrystals were synthesized via thermal decomposition of iron oleate. Magnetic cellulose particles were then fabricated using an oil-in-water single emulsion method. Enzymatic activity of cellulase on cellulose particles was assessed by using a hexokinase-based system to measure glucose release from cellulose particles after 2 hrs.

<u>In vitro study</u>: Magnetic cellulose particles at 2 mM Fe were treated with 1 mM *T.reesei/T.viride* cellulase (pH 5.0, 0.05 M acetic acid) for 3 days and used to make agarose gel phantoms at different [Fe]. r_2 and r_2^* relaxivity measurements were made at 4.7 T.

<u>In cellulo study</u>: MCF-7 human cancer cells were labeled overnight in media containing magnetic cellulose particles (1 mM Fe) and cellulase (1 mM) and used to make agarose gel phantoms. T_2 and T_2^* relaxation times were then measured at 4.7 T.

<u>RESULTS:</u> Cellulose particles were fabricated at a size of 414 ± 182 nm at 70% magnetite with r_2 and r_2^* molar relaxivity of 63 s⁻¹mM⁻¹ and 399 s⁻¹mM⁻¹ respectively. Cellulose nanoparticles dissolved to produce glucose when treated with cellulase enzyme at an initial rate that agreed with classic Michaelis-Menten kinetics (Fig. I). After 3-day treatment with cellulase, particles showed significant morphologic degeneration (Fig. IIa,b). Furthermore, the reaction mix of the cellulase-treated group was much darker than control (Fig. IIc). XPS spectra (Fig. IIa,b) showed a lower surface iron in the cellulase treatment group relative to control, suggesting higher dissolution of surface iron. Relaxometry of the two reaction mixtures showed a clear difference in r_2 relaxivity with the treatment group having 66% higher r_2 than control. The disparity in r_2^* relaxivity was not as large (Fig. III). Because r_2^* values are so much higher than r_2 for both treatment and control groups, we hypothesize that these particles exist in the static dephasing regime (where $r_2^* > r_2$).

Conclusion

These results demonstrate the potential of fabricating environmentally sensitive MRI contrast agents made entirely from a naturally occurring polymer. We believe that such agents could be used to report not only on cellular location, but also on intracellular events. For example, the differentiation of a transgenic neural progenitor cell line that expresses cellulase as a reporter gene when differentiating into a neuron could be detected via MRI, if labeled with a cellulosic agent like the one described here.

Clinical Relevance

Current MRI cell tracking technology only monitors the location of cells, providing no information on cellular fate. Key to any clinical implementation of MRI cell tracking is the ability to follow the viability and functionality of cells in vivo. By combining the highly magnetic properties of the Bangs particles and the biodegradability of Feridex[®] into an environmentally sensitive relaxation switch, these particles show the way forward to a more clinically adaptable cellular MRI.





Figure: I. Enzymatic activity of cellulase on cellulose particles. II. Morphology of cellulase-treated, magnetic cellulose particles. SEM/TEM/ XPS of cellulose particles incubated for 72 hrs (a) without, and (b) with 1 mM cellulase; (c) photo of cellulase-treated sample (right) with control (left). III. Relaxometric effect of cellulase treatment on magnetic cellulose particles. Measured (a) r_2 and (b) r_2^* molar relaxivities of particles following cellulase treatment. (c) Chart showing changes in r_2 (top) and r_2^* (bottom) molar relaxivities



Designed Hydrogen Bonding Driven Molecular Recognition at Interfaces in aqueous environment

Authors & Affiliations

Mingming Ma

Introduction

We designed, synthesized and studied a novel hydrogen bonding phospholipid system, using cyanuric acid (CA) and melamine (M) as the recognition motifs. Our study has confirmed that the selective heteromeric membrane apposition in aqueous solution was due to the molecular recognition between CA and M groups on the membrane surface. However, the major limitation of this system is that: the requisite density of CA/M modules for recognition was high. Trying to find the minimal valency required for the detectable lipid-lipid binding, we designed, synthesized and studied our second CA/M phospholipid system. Our studies have revealed that low membrane concentrations of the trivalent CA/M lipids or peptides retained the robust molecular recognition properties, although membrane activation upon binding was diminished.

Methods & Results

The robust molecular recognition of CA/M at the lipid-water interface promoted our efforts to extend the application to other apolar-water interfaces. As the first step, we still focused on the lipid-water interface. Instead of using functionalized lipids, we switched to an amphiphilic peptides: magainin. By covalently attaching the CA/M recognition motifs to magainin peptides, we found that these conjugates bound lipid membrane in a cooperative manner, and also their membrane activity was greatly enhanced. As a further step, we extended our interest to protein-protein interfaces. Streptavidin was chosen, since it is a well studied and widely used tetrameric protein. Synthesized biotin derivatives bearing the CA/M recognition motifs, and incorporated them onto streptavidin through biotin-streptavidin binding, we found that these functionalized streptavidin proteins could bind each other in a temperature-dependent and reversible manner.

Curious about the mechanism and driving force of the CA-M interaction in aqueous environment, we investigated the small molecule CA-M self-assembly in an aqueous solution with no interface. The CA-M self-assembly appears to be driven in large part by hydrophobic burial or "base-stacking" effects in water.

Conclusion

Overall, in five systems in aqueous environment, we have demonstrated the efficient molecular recognition directed by designed small molecule functional motifs, which could be used as novel adhesive materials and selective bio-marker in water.



Labeling of Porcine Mesenchymal Stem Cells with MRI Contrast Agent Ferex For Use In AAA Models

Authors & Affiliations

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Introduction

Mesenchymal stem cell (MSC) transplantation is being investigated in porcine abdominal aortic aneurysm (AAA) models for their potential to repair degenerated aorta. Reliable methods of labeling and tracking MSCs are necessary to allow non-invasive evaluation of their effects on aneurysms. Ferex is a superparamagnectic iron compound that when placed intracellularly can be identified in vivo by magnetic resonance imaging (MRI). This study aims to evaluate Ferex as an in vitro and in vivo cellular label in porcine AAA models.

Methods & Results

MSCs were isolated from pig bone marrow aspirates via Ficoll Paque separation and expanded in culture. MSCs were incubated with varying concentrations of Ferex and a cationic transfecting agent, Poly-L-Lysine (PLL). MSCs were then analyzed for cellular viability, phenotypic preservation and Ferex uptake. Transmission electron microscopy (TEM) was performed on individual Ferex-labeled MSCs. MRI was performed on Eppendorf tubes containing varying concentrations of Ferex-labeled MSCs suspended in 0.25 ml agar gel.

Ferex uptake was greatest with PLL and 200 ug/ml Ferex (2.7 ± 1.4 pg Fe/cell), with 87.1% cell viability. Flow cytometry analysis demonstrated that Ferex-labeled cells maintained phenotypic expression consistent with MSCs, with positive CD90 signals and negative CD45 and CD117. TEM confirmed that Ferex particles localized to lysosomes of labeled cells. There was a strong correlation (R2=0.9771) between the intensity of the MRI signal and the number of Ferex labeled cells.

Conclusion

As a result, these findings indicate that Ferex may be used as a labeling agent for in vivo tracking of transplanted MSCs in porcine AAA models.

Clinical Relevance

AAA is the 13th leading cause of death in the United States. Endovascular aneurysm repair (EVAR) has become the treatment of choice in most cases. However, EVAR does not eliminate the aneurysm. The risk of rupture is low following EVAR; nevertheless, the risk of rupture still exists. Therefore, close follow-up and frequent repeat imaging using CT angiography is necessary. Cell based therapy aimed at healing the damaged and degenerated aortic wall in AAAs may allow a definitive cure. This would eliminate the risk of rupture, reduce the need for follow-up and repeat imaging.





ml agar gel. A=10K cells, B=100K cells, C=500K cells and D=1,000K cells. The effect of the echo time on the signal and resultant T2* maps are shown.



Direct Comparison of Neck Pinhole Dual Tracer & Dual Phase Sestamibi Accuracies With & Without SPECT/CT for Parathyroid Adenoma Localization

Authors & Affiliations

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Introduction

There is uncertainty about accuracies of dual-phase (DP) Tc-99m sestamibi (MIBI) & dual tracer (DT, MIBI/I-123) parathyroid imaging protocols with the newly introduced SPECT/CT technique. Conversely, MIBI SPECT/CT was shown to be helpful in parathyroid adenoma (PA) localization (LOC), however, it may not have optimal resolution as pinhole (PH) particularly when PA is close to or within thyroid gland. This study compares PH DP & DT accuracies with & without (W/WO) MIBI SPECT/CT in primary hyperparathyroidism patients (PHP).

Methods & Results

155 PHP with histopathologically confirmed diagnosis (Dx) had PH DT/DP & early neck/chest MIBI SPECT/CT. PH DP W/WO SPECT/CT was reviewed for PA Dx & LOC by 2 observers. A separate review of PH DT W/WO SPECT/CT was performed. Confidences in PA Dx & LOC were scored as certain, probable or uncertain. Furthermore, PA was classified as clearly or unclearly distinguishable from thyroid gland. Contribution of delayed MIBI to PH DT was additionally scored as essential, confirming or non-contributory. Final Dx was classified as PA, parathyroid hyperplasia, & other/no pathology.

Of 153 confirmed PA, PH DT SPECT/CT was correct in Dx/LOC of 142 PA, significantly higher than PH DP SPECT/CT (104 PA). The ability to clearly distinguish PA from thyroid was higher in PH DT than DP W/WO SPECT/CT. Consequently, certainty of PA Dx was higher in PH DT than DP W/WO SPECT/CT. Certainty of PA LOC in both PH DT & DP were higher with than without SPECT/CT. In PH DT, delayed MIBI (DP) was essential for Dx/LOC of 24 uncertain PA.

Conclusion

In our large group PHP, accuracy & certainty of PA Dx/LOC were both higher in PH DT SPECT/CT than PH DP SPECT/CT. PH DT better identified PA than PH DP, while SPECT/CT improved certainty of PA LOC in both imaging protocols. In PH DT, delayed MIBI addition showed limited contribution & thus should be only considered if PH DT findings for PA Dx/LOC are uncertain.

Clinical Relevance

Parathyroid scintigraphy is considered the single best imaging modality for preoperative localization of PA. This study directly compares in a large group of patients with histopathologically confirmed diagnosis accuracies of the two different imaging protocols (DP, DT) as well as the two different imaging techniques (PH, SPECT/CT) used in parathyroid scintigraphy. The results of our study confirm the higher Dx accuracy of PH DT, which requires 1 hour less imaging time than PH DP with supplemental SPECT/CT imaging for high localization accuracy of PA.





Figure 1: A right inferior PA (red arrow) is discernible only on combined I-123 thyroid (A) and the following early MIBI image (DT) but in dual phase (DP, early/delayed) MIBI (B) pinhole Images. SPECT/CT images (C) help localizing PA site.



Figure 2: SPECT/CT MIBI images help localizing an anterior medisatinal parathyroid adenoma (red arrow).



CCR7 Targeted Nanoparticles for Molecular Imaging of the Regression of Atherosclerosis

Authors & Affiliations

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Introduction

Successful design of a molecular imaging contrast agent requires that in the tissue of interest there is a measureable difference between the retention of targeted and untargeted particles as a function of time. Molecular imaging agents for atherosclerosis must be able to penetrate the arterial endothelium, and to interact with specific cells in the plaque. CCR7 is a chemokine receptor expressed by immune cells that has been shown to be upregulated in regressing atherosclerotic lesions relative to progressing lesions *in vivo*. Molecular targeting of this receptor could be used to aid in diagnosis, and to monitor response to treatment. We previously showed by a fluorescent competition binding assay that 19N7, a peptide composed of the 7 amino acids of the N-terminus of CCL19—the native ligand to this receptor—exhibits equal binding affinity to the ligand. To investigate the feasibility of molecular imaging of regressing plaque, we used 19N7 to functionalize a multimodal nanoparticle MRI contrast agent with a size and composition similar to that of native high-density lipoprotein (HDL). In addition to physical structure and molecular relaxivity, particles were characterized for the biological properties of binding affinity and receptor specificity, their ability to permeate cultured endothelial cell monolayers, and their rate of uptake by a cell line expressing an artificial CCR7 construct, and against a native CCR7 expressing cell line.

Methods & Results

Binding experiments were conducted against HEK-293 cells transfected with a GFP-labeled CCR7, and the mouse dendritic cell line DC2.4. Endothelial permeability assays were conducted with bovine aortic endothelial cells. Native structure of the transfected protein was confirmed by colocalized binding of fluorescently labeled CCL19 to the extracellular domain of GFP-CCR7 (Fig. 1). Oleic-acid coated iron-oxide nanocrystals were used as the foundation material for construction of lipoproteinlike nanoparticles, synthesized as described previously by Cormode et al. (Nano Lett (2008) 3715-3723). Binding specificity and particle uptake kinetics were determined by measuring the fluorescence of rhodamine labeled phospholipids incorporated into experimental particles, containing 19N7-conjugated phospholipids, or control particles. Fig. 2 shows the two variants of the nanoparticles we created: A) using a phospholipid composition stabilized with apoplipoprotein A-1, similar to that of native (HDL); B) the same, with 19N7; C) a "stealth" formulation using polyethylene glycol 2000 phosphatidyl ethanolamine as the stabilizing agent; and D) the stealth particle with 19N7. Particle size was determined by dynamic light scattering and TEM, and found to be similar to native HDL, and endothelial permeability was found to be intermediate between that of albumin and LDL (Fig. 3). Non-specific binding of both targeted and untargeted particles was identical. The concentration corresponding to 50% receptor saturation was 4 times lower for targeted particles than for untargeted (Fig. 4).

Conclusion

We show that lipoprotein based MRI contrast agents functionalized with 19N7 have the requisite properties to both penetrate arterial plaque, and to interact with cells of a single phenotype but differing expression of CCR7.





Figure 1) Human embryonic Kidney cells (HEK-293) expressing the fluorescently labeled mouse receptor GFP-CCR7 (Green) incubated with Alexa-532 labeled CCL19 (Red) at 0.2 nM for 15 minutes. Colocalization of the ligand and receptor appears yellow. Scale bar 20um.

Figure 2) TEM images of lipoprotein based iron-oxide core nanoparticles. A) Fe-HDL: Untargeted HDL-like particles coated with 1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine (MHPC), and stabilized with ApoA-1. B) Fe-HDL-19N7: as in A with targeting ligand C) Fe-PEG: untargeted particles coated with MHPC and PEG-2000-PE. D) Fe-PEG-19N7: as in C, with targeting ligand. Scale bar 50nm.

Figure 3) Permeability of cultured endothelium to nanoparticles was measured by detection of the increase in fluorescence in the abluminal chamber of a pressure flow apparatus. Experimental particles are shown in red, size standards are shown in blue. The dotted line represents convective flux as directly proportional to the inverse of diameter.

Figure 4) The increase in fluorescence of cultured DC2.4 cells activated with an LXR agonist to upregulate CCR7 measured as a function of time. Targeted cells are taken up more rapidly and show a more distinct saturation plateau.

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Nanoclusters of iron oxide: effect of core composition on structure, biocompatibility and cell labeling efficacy

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Introduction

Some of the best methods for synthesizing inorganic nanocrystals yield hydrophobic nanoparticles, so methods to make these nanocrystals biocompatible are of prime importance. Biocompatibility is crucial for cell tracking, where large quantities of diagnostically active nanoparticles are loaded into cells to allow their visualization with non-invasive imaging techniques *in vivo*. In this report, we studied the effect of six core compositions (soybean oil, MCT, cottonseed oil, corn oil, olive oil and no oil) on the structure of lipid coated iron oxide nanoclusters, cell labeling efficacy and cell viability, with the aim of determining an optimal formulation to be used in cell tracking.

Methods & Results

Fluorescently labeled lipid-coated iron oxide nanoclusters were prepared using a solvent evaporation technique, as described by Jarzyna *et al.* (Biomaterials, 2009, 6947-6954). Nanoparticle size and morphology (A) were studied with dynamic light scattering and transmission electron microscopy (TEM). Relaxivities (r_1 and r_2) were acquired at 9.4 T. Murine macrophage cells (RAW 264.7) were incubated with each nanoparticle at a final concentration of 200 µg Fe/ml for 3 h at 37 °C. Cell uptake was evaluated with using TEM as well as fluorescence- and light microscopy (B-D). The contrast in the cells was determined by MR imaging (E) and cell viability was probed using the MTT assay (F).

An initial screening process found that the nanoclusters produced by cottonseed oil, corn oil and olive oil to lead to low cell viability. High payloads of hydrophobic iron oxide nanocrystals were incorporated into the nanoparticles with each type of core used (A). The macrophages took up large quantities of nanoparticle (B-D), with the iron-oxide only nanoparticles taking up the most (4.7 pg Fe/cell). MR images of cells incubated with nanoparticles corroborated this result with most contrast (signal loss) observed with iron-oxide only nanoparticles (E). The MTT assay revealed that the iron-oxide only nanoparticles reduced the viability of cells the least with increasing toxicity observed from MCT core and soybean oil core nanoparticles (F). Furthermore, while storage under nitrogen or air made no difference to the effect on cell viability for MCT core or iron-oxide only nanoparticles, storage under nitrogen reduced the toxicity of the soybean oil core nanoparticles formulations increased as the time since synthesis increased (F), potentially due to oxidation of the triglycerides in the soybean oil.

Conclusion

Clearly, even when combining biocompatible materials such as soybean oil and iron oxides, the resulting formulation is not guaranteed to be biocompatible. The formulation without oil caused the lowest toxicity and greatest cell labeling (4.7 pg Fe/cell) and hence is the best candidate to be used in cell tracking applications.

Clinical Relevance

With the cessation of production of clinically approved iron oxides such as Feridex, the herein reported lipid-coated nanoclusters represent viable alternatives for cell tracking applications.







Collagen-targeted High Density Lipoprotein Nanoparticles for Evaluation of Plaque Regression by Molecular Magnetic Resonance Imaging

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Introduction

Reconstituted high density lipoprotein (rHDL) interact with macrophages naturally, which have been modified to serve as a molecular magnetic resonance (MR) imaging contrast agent (1, 2). The Reversa mouse model (LDLR^{-/-}ApoB^{100/100}Mttp^{fl/fl}Mx1-Cre^{+/+}) showed decrease of macrophage but increase of collagen contents under regression conditions(3, 4). The rHDL nanoparticles were conjugated with collagen targeting peptide (EP3533) labelled with Gd-DTPA-DSA and Rhodamine. In this study we evaluate these nanoparticles for in vivo non-invasive molecular MR imaging of the atherosclerotic plaque regression in Reversa mouse model.

Methods & Results

Collagen specific EP3533 peptides were conjugated to rHDL nanoparticles (EP3533-HDL) through PEG-lipids via EDC method. EP3612 peptides contain a D-Cys, a non-natural amino acid, in comparison with EP3533 peptides. EP3612 conjugated rHDL nanoparticles were used as non-specific control (EP3612-HDL). The binding of nanoparticles to collagens and some other extracellular matrix components were examined *in vitro* coated plates using fluorescence emission. EP3533-HDL exhibited significantly higher binding to collagens as compared to HDL and non-specific EP3612-HDL (Fig 1).

The Reversa mice were used to evaluate the *in vivo* efficacy of the nanoparticles to MR image atherosclerotic plaque regression that was induced by reducing plasma lipid levels (Fig. 2). At Day 0, the collagen-targeting EP3533-HDL nanoparticles did not enhance the signal of vessel wall. At Day 28, only collagen-targeted rHDL-3533 caused MR enhancement of the vessel wall at 24 h post injection. Under progression condition, the MR imaging of vessel walls kept the same at Day 28 as at Day 0 for EP3533-HDL. On the other hand HDL or EP3612-HDL did not lead to enhancement in MR images of the vessel wall after regression.

Ex vivo confocal microscopy revealed that EP3533-HDL nanoparticles were colocalized with collagen type I rich area in the atherosclerotic plaques at Day 28 under regression conditions. Immunohistostaining showed significant decrease of CD68+ macrophage positive area and increase of collagen positive area inside plaques in aortic vessel walls at Day 28 regression, while there were slight change of macrophages and collagens at Day 28 progression control group.

Conclusion

We demonstrated that conjugation of collagen-targeting peptides to HDL nanoparticles increased binding to collagens in vitro. In vivo MR imaging revealed that rHDL-EP3533 nanoparticles significantly enhanced the signal of collagen-rich plaques, which allows the non-invasive visualization of plaque regression.





Fig.1. *in vitro* binding of nanoparticles to different types of collagens and other extracellular matrix (ECM) components coated on plates.



Fig.2. Typical MR imagines of aortic vessel walls of Reversa mice under (A) regression and (B) progression conditions. The mice were injected with 50 μ mol/kg Gd of HDL, EP3533-HDL, or EP3612-HDL. The arrows point to aortas.



Quantum dot and Cy5.5 labeled nanoparticles to investigate lipoprotein biointeractions via Förster resonance energy transfer

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Introduction

The study of lipoprotein biology in general and high density lipoprotein's (HDL) biointeractions in particular is of primary importance to better understand, treat and prevent cardiovascular disease. Hybrid nanostructures based on nanocrystals that are stabilized and functionalized by a biocompatible coating have been exploited for various imaging techniques and are specifically suitable for molecular imaging purposes. In this study, we developed a quantum dot core HDL nanoparticle. By detecting Förster resonance energy transfer (FRET) between the quantum dot (QD) core and dye-labeled lipids in the coating, we are able to study lipid exchange dynamics, lipoprotein-lipoprotein interactions, and to visualize the process of HDL uptake by live macrophage cells.

Methods & Results

Exceptionally stable CdSe/CdS/ZnS core-shell-shell (CSS) QDs were synthesized and coated with phospholipids. A certain fraction of lipids were labelled with Cy5.5 dyes. Subsequently, apolipoprotein A-I (apoA-I) was incorporated into the lipid corona, after which the product was purified to obtain single QD core and Cy5.5 labeled HDL-like nanoparticles (QD-HDL-Cy5.5) (Figure 1a). Both emission spectra and fluorescence decay curve confirmed the occurrence of FRET and revealed that the FRET efficiency depends on the amount of Cy5.5 in the lipid corona. (Figure 1b,c) Our QD-HDL-Cy5.5 is very well suited to study lipid-exchange dynamics of inter-lipoproteins and also of lipoproteins and living cells, since lipid-exchange induces changes in the areas under the emission spectra of the QD and Cy5.5 (Figure 2a). The faster exchange of nanoparticles without apoA-I confirmed its stabilizing features (Figure 2b). Finally, we performed a proof-of-principle study to visualize the temporal fate of QD-HDL-Cy5.5 once associated with living J774A.1 macrophages. Using fluorescence microscopy, we were able to observe the QD core, Cy5.5 lipids and FRET, respectively. We found the fluorescence from the lipids to mostly originate from the cell membrane, while the QD core was predominantly found in cytoplasm, suggesting a dissociation of HDL upon association with macrophage cells.

Conclusion

We developed a new hybrid nanostructure resembling HDL, containing a QD core and Cy5.5 labelled lipids as FRET pairs. We show that this nanoprobe is a powerful tool to study lipid-exchange dynamics of lipoproteins and also to visualize the incorporation process of HDL by macrophages cells.





Figure1. (a) Schematic representation of a quantum dot core and Cy5.5-lipid dual labeled HDL nanoparticle. (b) Emission spectra of QD-HDL with varying amounts of Cy5.5-lipid for excitation at 406 nm. (c) Fluorescence decay curves of the QD emission (620 nm, excitation at 406 nm) for QD-HDL with varying amounts of Cy5.5-lipid.



Figure 2(a) Schematic representation of the QD-HDL-Cy5.5 exchanging Cy5.5-lipids with the cell membrane and the effect on FRET. (b) ICy5.5/IQD of QD-HDL and QD-micelle samples that were mixed with THP-1 macrophage cells at different temperatures.

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Abstracts, Cancer Imaging category





Intravoxel Incoherent Motion Diffusion Imaging of the Liver: Initial Experience

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Introduction

DW-MRI provides non-invasive in vivo quantification of the combined effects of capillary perfusion and diffusion without the use of exogenous contrast media. The intravoxel incoherent motion (IVIM) model has rarely been described in liver applications. This study is to describe the preliminary experience with the use of monopolar vs. bipolar diffusion gradients for IVIM DW-MRI of the liver.

Methods & Results

Free breathing (FB) and respiratory-triggered (RT, using navigator echo) coronal single shot EPI IVIM DW-MRI was performed in 9 initial subjects [4 healthy volunteers and 5 patients with hepatitis C virus infection (HCV)] at 1.5T (Siemens Avanto) using monopolar and bipolar diffusion gradients. Sequence parameters were: TR = 1 respiratory cycle for RT and 3000 for FB, TE = 63 (monopolar) and 73 (bipolar), 16 b-values (0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 175, 200, 400, 600, 800 s/mm²), voxel size 2.6 x 2.1 x 8 mm, 2 averages, fat suppression (SPAIR), GRAPPA2. Two observers assessed liver edge delineation and distortion/ghosting on the four sequences (bipolar RT vs. bipolar FB vs. monopolar RT vs. monopolar FB, max score per patient=36). A single observer measured SI (signal intensity) and SD of noise at all b-values in liver parenchyma (6 ROIs in right posterior lobe). A bi-exponential diffusion model was applied to calculate hepatic perfusion fraction (PF), the pseudo-diffusion coefficient (D*), and the true diffusion coefficient (D) using MatLab. ADC was calculated using all b-values. Image quality, SNR, IVIM parameters, and goodness of fit were compared between the 4 sequences.

The RT sequence showed less blurring compared to FB sequence, and bipolar sequence showed less distortion and ghosting compared to monopolar sequence, with trend towards significance for both (p=0.059). No significant differences in PF, D*, D and ADC values were present when comparing RT vs. FB sequences and monopolar vs. bipolar techniques (p=0.16-0.99).The best/worst goodness of fit was observed for RT bipolar/FB monopolar sequences (root mean square error 1.75 vs. 3.17). PF was lower in HCV patients (p=0.034), D* and ADC were lower in HCV patients without reaching significance.

Conclusion

In our initial experience, we found slightly better image quality with RT compared to FB acquisition, and better fit for bipolar diffusion acquisition, although the IVIM parameters were equivalent between all sequences. PF is a promising marker of liver fibrosis.

Clinical Relevance

Artifacts affecting DW-MRI can significantly degrade image quality and affect ADC and IVIM calculations. Respiratory triggered and bipolar diffusion acquisition showed the best image quality, this should be further confirmed in larger data sets.





Figure 1: Coronal voxel-based parametric map of IVIM perfusion fraction (PF) from a healthy volunteer (left) and an HCV-patient (right).



Figure 2: IVIM diffusion decay curves shown in a healthy volunteer (left) and an HCV-patient (right)



Evaluation of Fluorescent Deoxyglucose as a Topical Contrast Agent to Detect Colonic Neoplasia Using Confocal Imaging.

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Introduction

The emerging role of confocal microendoscopy has generated interest in identification of contrast agents that can be used to distinguish neoplastic from non-neoplastic gastrointestinal mucosa. Currently available agents have significant limitations in both specificity and accuracy. This study evaluates 2-NBDG ((1-[N-(7-nitrobenz-2-oxa-1, 2-diazol-4-yl)amino]-2-deoxy-D-glucose), a fluorescently labeled deoxyglucose, in the identification of colonic dysplasia and neoplasia using confocal microscopy.

Methods & Results

Biopsies from subjects with varied histological grades of colonic dysplasia (both polypoid and flat) were incubated *ex vivo* at 37°C with 2-NBDG and imaged with a fluorescence confocal microscope. The average glandular mean-flourescence intensity was calculated as marker of dysplasia and resulting grades compared with histological interpretation as the gold standard. A significantly higher level of mean-fluorescence intensity was evident in dysplastic (130.3±12.0) epithelium as compared to both histologically normal (67.1±23.2) and hyperplastic polyps (62.2±24.8). When present, chronic inflammation was associated with increased 2-NBDG uptake in the lamina propria but not the glandular epithelium and did not affect the overall mean-flourescence

Conclusion

2-NBDG shows promise as a topical contrast agent for confocal imaging as shown by its ability to delineate biochemical and morphological features of dysplasia and neoplasia. Further testing, *in vivo*, is needed to determine its performance during confocal microendoscopic imaging.

Clinical Relevance

intensity observed.

Early detection of neoplasia has significant implications for surveillance, treatment and survival. Clinicians ability to visualize precancerous lesions and early dysplasia during the endoscopic exam has the potential to alter immediate patient management. There is a need for fluorescent contrast agents that can enhance diagnostic accuracy during confocal endoscopic imaging of the gastrointestinal tract for colorectal cancer screening and surveillance.





Sample confocal images of biopsies stained with 2-NBDG and imaged using identical settings. A. Normal colon mucosa, B.Hyperplastic polyp, C. Tubular adenoma, D. Mean intensities of all identifiable glands as measured with imaging software (mean±SD).



The Use of the High-Resolution Microendoscope (HRME) for Intraoperative Detection of Cancer: Preliminary Ex Vivo Diagnostic Accuracy and Inter-rater Reliability

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Introduction

The overall five-year survival for advanced stage HNSCC is a dismal 26%, and the efficacy of surgical treatment depends critically on obtaining negative margins of resection. While intraoperative "frozen section" analysis of surgical margins is a valuable adjunct to ablative surgery, the method is expensive, time-consuming, and highly dependent on the expertise of the pathologist. Thus, non-destructive optical methods which can image the interface between normal and cancerous tissue in real time have the potential to improve the accuracy of surgical resection and reduce the number of frozen section analyses. The aim of this study was to determine if physicians experienced with the care of head and neck cancer can accurately discriminate between images of cancerous and benign mucosa obtained with the high-resolution microendoscope (HRME) during the course of standard-of-care surgical resection.

Methods & Results

Forty-four patients at the Mount Sinai Hospital diagnosed with HNSCC were prospectively enrolled in this study. Specimens were imaged *ex vivo* with the HRME after treatment of resected tissue with the nuclear contrast agent proflavine (0.01%). Imaged sites were concurrently evaluated with conventional histopathologic analysis of punch biopsies, resulting in 44 high-quality HRME images with a pathologic diagnosis. These images were then used to generate training and testing sets to measure the diagnostic accuracy of contrast-enhanced HRME and determine inter-rater reliability. Seven physicians experienced in the care of patients with head and neck cancer were administered a 37-image test set (11 normal, 26 dysplasia and cancer) after training with seven representative images.

The overall accuracy of contrast-enhanced HRME in diagnosing normal (benign) vs. abnormal (dysplastic or cancerous) mucosa was 97 percent (95% CI = 94-99 percent). The mean sensitivity and specificity was 98 percent (95% CI = 96-100 percent) and 92 percent (95% CI = 84-100 percent), respectively. The overall mean negative predictive value was 96 percent (95% CI = 94-100 percent). The kappa statistic for inter-rater reliability was 0.90 (95% CI = 0.88-0.93) among all physicians.

Conclusion

The HRME is a promising technique that may ultimately provide real-time histopathologic assessment of surgical margins and decreased utilization of frozen section analysis in the head and neck.

Clinical Relevance

In the surgical resection of head and neck cancer, it is imperative to obtain clean surgical margins while removing as little normal tissue as possible, but the current method of the use of frozen sections for this purpose is flawed. High-resolution microendoscopy (HRME) provides a real-time optical imaging alternative that may allow for more targeted use of frozen sections, while giving the surgeon enhanced visualization of cancerous tissue in the operating room.



Figure 1. The High-Resolution Microendoscope (HRME). A 1 mm diameter fiber optic probe is shown extending from the device below, which is placed on the tissue surface to obtain images. A 445 nm LED light provides illumination. The device is small enough to fit in a standard briefcase, and is connected to a laptop to record the images, which are viewed in real time.



Figure 2. Representative HRME Images. A) Benign tongue mucosa as seen on HRME. The white dots represent nuclei, and a regularly spaced and equally sized, indicative of benign tissue. The corresponding histopathology is shown on the right. B) Squamous cell carcinoma of the tongue, as seen on HRME. The white dots again represent nuclei, and are enlarged and show a loss of tissue architecture, indicative of cancer, as shown on the corresponding histopathologic image on the right.





Spatial Profiling of Anticancer Drug in Brain Tumor Tissue using MALDI Mass Spectrometric Imaging

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Introduction

Gefitinib is a competitive tyrosine kinase inhibitor (TKI) targeting the epidermal growth factor receptor that disrupts key signaling pathways affecting cell growth. It is used to treat different types of tumors including brain tumors that are known to be very invasive and heterogeneous in nature. Several analytical methods have been applied, such as liquid chromatography mass spectrometry (LC-MS), to measure gefitinib concentration in whole tissue homogenates; however spatial information is lost as samples are homogenized before analysis. Matrix-assisted laser desorption ionization (MALDI) mass spectrometric imaging (MSI) offer the high sensitivity and selectivity of mass spectrometry while maintaining the physical tissue structure and could lead to a better understanding of the determinants of drug distribution and its variability within tumors.

Methods & Results

Mouse brain tumors were excised from drug treated and untreated mice bearing intracerebral tumors and 12 μ m frozen sections were collected using a microtome. Without further treatment, tissue sections were placed on ITO-coated microscope slides from Laser BioLabs and stored at -80 °C until further use. The sections were thawed to room temperature before applying MALDI matrix solution using the inkjet printer method. The slides were loaded into an AB Sciex 5800 TOF/TOF and the 4800 Imaging (maldi-msi.org) software was used for MSI data acquisition. BioMAP v3.8.0.3 from (maldi-msi.org) was used to process the acquired data. Immunohistochemical (IHC) results of adjacent serial sections were compared with the MSI data.

Sections of brain tumors were selected for MSI analysis using full MS scan. Initial extracted ion images (EII) of a molecular ion (M+H⁺, m/z 447), and fragment ion (m/z 296) of gefitinib were successfully generated with good signal-to-noise ratio. These EII's were normalized against total ion count (TIC) to correct matrix distribution difference. Adjacent tumor sections were also processed for IHC analysis of various biomarkers such as microvessel density that will be correlated to EII images. The initial normalized ion image of gefitinib showed preferential distribution to the tissue border. The tumor heterogeneity, in part due to breakdown of the blood-brain barrier, could influence drug distribution and the associated pharmacodynamic actions of gefitinib. Both the intra-tumor and intertumor distribution of gefitinib will be sought to gain an understanding of its heterogeneity and determinants.

Conclusion

Although mass spectrometric imaging (MSI) is an invasive, destructive and relatively low resolution imaging technology, it can be used to study molecular spatial profile either targeted or nontargeted. With the development of suitable internal standard and sample preparation methods, quantitative measurement of molecule of interested can be achieved. In study drug pharmacokinetics, MSI can provide drug spatial distribution data in addition to data collected through other methods without using labelling.

Clinical Relevance

Currently, MSI is mainly used in experimental animal tissue imaging and clinical pathologic specimen analysis. Potentially, MSI can be applied to clinical autopsy sample analysis.





Figure 1. Comparison of selected marker immunohistochemistry fluorescent staining imaging and mass specrometric imaging of Gefitinib. (A) IHC of microvessel marker, CD31 (red color); apoptotic cells marker, TUNEL (green color); and pericyte marker, alpha-SMA (blue color). (B) MSI of molecular ion ($M+H^+$, m/z 447) of Gefitinib.



Figure 2. (A) Four different areas were selected to compare Gefitinib distribution. (B) Representative mass spectrum of area 1. (C) Representative mass spectrum of area 4.



Development of a combined photoacoustic micro-ultrasound system for non-invasive detection of mouse lymph nodes and intratumoral oxygen saturation measurements

Authors & Affiliations

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Introduction

• Photoacoustics (PA) is an imaging modality which combines the sensitivity of optical imaging with the low acoustic scattering and resolution of micro-ultrasound (μ US). The differing optical absorption spectra of oxygenated and deoxygenated hemoglobin (Hb) (Fig 1) make photoacoustics an ideal modality for in vivo imaging of oxygen saturation (sO2).

Methods & Results

A photoacoustic imaging system (Vevo LAZR, VisualSonics, Toronto, Canada) was operated whereby light was generated by a tunable laser (680 - 970 nm) and delivered through fiber optic bundles integrated into a linear array transducer (LZ-250, fc = 21 MHz). The jugular vein of a Sprague dawley rat was imaged. Measured sO2 values were compared to predicted values obtained from an online calculation based on GR Kelman (1966). Xenograft Lewis lung carcinoma (LLC) tumors in nude mice were imaged in 2D.

Serial dilutions of MB were imaged with PA at 680 nm (peak absorbance, Fig. 5) by drawing the solution into a 500 μ m diameter polyethylene tube.

PA images of the axillary lymph node were collected in 2D and 3D at 680 and 760 nm both pre- and post-infusion of MB into the forepaw of adult CD1 mice (n=3). A multispectral image was generated by merging a colorized (blue) subtraction image (760 nm image subtracted from the 680 nm image) with the image acquired at 760 nm using ImageJ image processing software (NIH, Bethesda, USA). Axillary and brachial lymph nodes were excised and imaged to verify the source of the MB signal.

• All animal procedures were performed under animal care committee approved protocols according to federal animal care guidelines.

Conclusion

Changes in pO2 values and sO2 values correlated well with alterations in inhaled O2 concentration (data not shown).

• A significant correlation between the measured and predicted values was observed (Fig 2) (R2= 0.832, P<0.01).

• Changes in inhaled oxygen concentration resulted in corresponding changes in tumor oxygen saturation in non-hypoxic but not in hypoxic regions

Clinical Relevance

Potential applications include the localization and determination of hypoxic states within a tumor (Fig 3) or other tissue and how it may vary with experimental treatments. The sensitivity of the system with regard to methylene blue has been demonstrated and the in vivo imaging of lymph nodes has implications for metastasis research.

The results obtained here with a combined $PA/\mu US$ system show that data can be obtained relating to tumor microenvironment. The use of this system in conjunction with nanoparticle technology may provide further developments including imaging of molecular processes.



