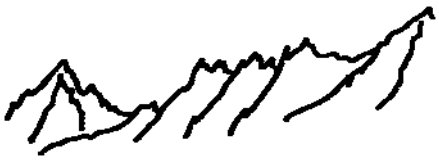
TOPIM '07



HOT TOPICS IN MOLECULAR IMAGING
FEBRUARY 19 – 23, 2007
ECOLE DE PHYSIQUE, LES HOUCHES, FRANCE

Imaging in neuroinflammation and neurodegeneration

SCIENTIFIC COMMITTEE

Dr. Bertrand Tavitian
Dr. Andreas Jacobs
Dr. Gitte Moos Knudsen
Dr. Nicole Deglon

esmi
European Society for Molecular Imaging
European Molecular Imaging Laboratories
DiMI

	Monday
08:00	BREAKFAST
08:45	
SESSION	**New PET Tracers in neuroinflammation**
09:00 - 09:40	**INTRODUCTION** **Bertrand Tavitian, President ESMI**
09:40 - 10:20	**Binding to the Peripheral Benzodiazepine Receptor in the Human Brian using PET and Autoradiography** Christer HALLDIN, Balázs GULYÁS,Boglárka MAKKAI,Péter KÁSA,Jan ANDERSON,Kazatoshi SUZUKI,Tetsuya SUHARA - Karolinska Institutet, Stockholm, Sweden
10:20 - 10:40	
10:40 - 11:20	**Carbon-11 and fluorine-18 labelled radioligands for imaging the peripheral benzodiazepine receptors** Frederic Dollé, CEA , Orsay, France
11:20 - 12:00	**Challengers of PK11195** Hervé Boutin, University of Manchester, Manchester, UK
12:00 -12: 25	Presentation submitted abstract: **PET Imaging of PBR in a rat model of HSV encephalitis** Doorduin J, Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, The Netherlands
12:30	LUNCH
13:00	
13:30	
14:00	DIMI Joint WP3/9 meeting
15:00	
16:00	
18:00	
SESSION	**Molecular Imaging of NeuroInflammation**
18:00 - 18:40	**Quantifying [11C]PK11195 uptake in HIV dementia** Chris Endres and Martin Pomper, John Hopkins University, USA
18:40 - 19:20	**New Tracers for Translocator Protein (18 kDa) (TSPO) Exploration by PET and SPECT: Preclincal Studies** Sylvie Chalon, A. Katsifis, M. James, N. Arlicot, F. Mattner, R. Banati, S.Meikle, J. Vercoullie, D. Guilloteau, M. Kassiou - University of Tours, Tours, France
19:30	DINNER
20:00	
20:30	
20:30 - 21:00	Presentation submitted abstract: **Evaluation of compartiment models and semi-quantitative measures of PET brain studies** Ronald Boellaard VU University Medical Centre, Dep.of Nuclear Medicine & PET Research, Amsterdam, The Netherlands.

	Tuesday
08:00 08:45	BREAKFAST
SESSION	**Molecular Imaging of NeuroInflammation**
09:00 - 09:40	**PK11195 as a marker of inflammation in model of stroke** Anna Planas, IDIBAPS, Barcelona, Spain
09:40 –10:20	**PET and molecular imaging in preclinical models of inflammation and neurodegeneration** Sara Belloli, IRCCS San Raffaele, Milano, Italy
10:20 - 10:40	BREAK
10:40 -11:20	I**maging neuronal and microglial activation during seizues in genetically modified mice** Martine Mirrione, Molecular and Cellular Pharmacology, Brookhaven NY, USA
11:20 - 12:00	**Visualization of micro- and astroglia in health and disease states by two-photon laser microscopy** Frank Kirchhoff, Max Planck Institute of Experimental Medicine, Göttingen, Germany
12:00 -12: 25	Presentation submitted abstract: **A study of immunological response to brain ischemia using FMT** Juan Aguirre, IESL – FORTH, HERAKLION, GREECE
12:30 13:00 13:30	LUNCH
14:00 15:00 16:00 17:00	
17:20 **SESSION**	**EARLY START: 17:20** **Molecular Imaging of Neuroinflammation and Neurodegeneration**
18:00	**Is the Vesicular Acetylcholine Transporter (VAChT) a Good Target for Neurodegenerative Disease?** Michael Kassiou, M. Allard, J. Mazère, N. Giboureau, J. Vergotte, P. Emond, D. Guilloteau - Brain and Mind Research Institute, University of Sydney, Australia
18:00 - 18:40	**Basic aspects of inflammation in CNS bacterial infections and potential target candidates for imaging** Kielian Tammy, University of Arkansas for Medical Sciences College of Medicine, USA
18:40-19:30	DRINK OFFERED BY PHYSICS SCHOOL
19:30 20:00 20:30	DINNER
20:30 - 21:00	Presentation submitted abstract: **PET IMAGING OF PBR IN A RAT MODEL OF HSV ENCEPHALITIS** Erik F.J.De Vries, University Medical Center Groningen, Groningen, the Netherlands.

	Wednesday
08:00 08:45	BREAKFAST
SESSION	**Molecular Imaging of neurodegenerative diseases - focus on Alzeihmer disease I**
09:00 – 09:40	**Phenotyping of mouse models of Alzheimer's disease by PET** Alexandra Winkeler & Andreas Jacobs, Max Planck institute Cologne, Germany
09:40 – 10:20	**Phenotyping of mouse models of Alzheimer's disease by MRI: Anatomy, amyloid and functional imaging.** Marc Dhenain, Nadine El Tannir El Tayara, Andreas Volk, Benoît Delatour, CEA, Orsay, France
10:20 - 10:40	BREAK
10:40 – 11:20	**Analysis of neurodegenerative and neuroinflammatory changes upon locus ceruleus cell death in transgenic mouse models of Alzheimer`s disease** Michael Heneka, university of Muenster, Muenster, Germany
11:20 – 12:00	**Development and preclinical evaluation of a fluorine-18 labelled tracer for Alzheimer's Disease** Van Laere Koen and Alfons Verbruggen, Nuclear Medicine Department Leuven University Hospital, Belgium
12:00 – 12: 25	**2-(3'-[11C]methylamino-4'-aminophenyl)-1,3-benzothiazole** Kim Serdons,Nuclear Medicine Department Leuven University Hospital, Belgium
12:30 13:00 13:30	LUNCH
14:00 15:00 16:00 17:00 18:00	
SESSION	**Molecular Imaging of Neuronal diseases**
18:00 -18:40	**Neuroimaging Studies in an Animal Model of Inhalant Abuse and incorporate behavioral models of addiction, structural imaging with microMRI and functional imaging with microPET using several new ligands as well as FDG** Wynne Schiffer, Molecular and Cellular Pharmacology, USA
18:40-19:10	Presentation submitted abstract: **New approach for measuring P-glycoprotein in the blood-brain barrier, synthesis and biodistribution of [11C]Laniquidar.** Luurtsema G Department of Nuclear Medicine and PET Research, VU University Medical Center, Amsterdam, The Netherlands.
19:30 20:00 20:30	DINNER
20:30 - 21:00	

	Thursday
08:00	BREAKFAST
08:45	
SESSION	**Molecular Imaging of Neurodegenerative diseases**
09:00 – 09:40	**MOLECULAR AND STRUCTURAL NEUROIMAGING IN CEREBELLAR ATAXIA** Elena Salvatore, Department of Neurological Sciences, University of Naples, Naples, Italy
09:40 - 10:20	**Amyloid Plaque Imaging in Non-Alzheimer Dementia** Drzezga Alex, KrdI, TU-München, München, Germany
10:20 - 10:40	BREAK
10:40 - 11:20	**MRI measures of inflammation and degeneration in Multiple Sclerosis** Mara Rocca, Neuroimaging Research Unit, San Raffaele Hospital, Milan, Italy
11:20 - 12:00	"**q-Space Analyzed Diffusion Weighted MRI in Multiple Sclerosis**" Henrik Lund, Copenhagen University Hospital, Copenhagen, Denmark
12:00 -12: 25	Presentation submitted abstract **Metabolic-dopaminergic mapping of the Quinolinic Acid rat model for Huntington's Disease** Casteels Cindy , Nuclear Medicine Department Leuven University Hospital, Belgium
12:30	LUNCH
13:00	
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18:00	
SESSION	**Therapeutical applications in Molecular Imaging** **& Molecular Imaging of Neuronal diseases**
18:00 - 18:40	**A VIEW OF LIGHT BY MRI : PHOTOCHEMICAL AND HEMODYNaMIC CHANGES INDUCED DURING VASCULAR-TARGETED PHOTODYNAMIC THERAPY (VTP)** Yoram Salomon, Departement of Biological Regulation Weizmann Institute of Science, Israel
18:40-19:20	**Assessment of therapy after cerebral ischemia by MRI** Uwe Himmelreich, Max Planck Institute Cologne, Cologne, Germany
19:30	"SOIREE RACLETTE"
20:00	DRINK OFFERED BY ORGANISATION
20:30	
20:30 - 21:00	Presentation submitted abstract: **Multi-modal imaging of functional brain activation n- methodological evaluation and preliminary results** Backes H. Department of Neurology at the University of Cologne, Cologne, Germany

	Friday
08:00 08:45	BREAKFAST
SESSION	**Therapeutical applications in Molecular Imaging and future perspectives**
09:00 – 09:40	**Imaging of neurodegeneration and stem cell migration in rodent models using lentiviral vectors** Abdelilih Ibrahimi, Neurobiology and Gene Therapy, Leuven, Belgium
09:40 – 10:20	**Gene transfer and imaging in the CNS** Deglon Nicole, CEA, Orsay, France
10:20 - 10:40	BREAK
10:40 – 11:20	**New horizons for metabolic, functional and molecular imaging of the brain** Rolf Gruetter EPFL, Lausanne , Switserland
11:20 – 12:00	**Visualisation of dendritic cells and their progenitors by MRI** Uwe Himmelreich, Max Planck Institute Cologne, Germany
12:00 – 12: 25	**FAREWELL, Bertrand Tavitian, President ESMI**
12:30 13:00 13:30	LUNCH
END OF THE MEETING	

BINDING TO THE PERIPHERAL BENZODIAZEPINE RECEPTOR IN THE HUMAN BRIAN USING PET AND AUTORADIOGRAPHY

Christer Halldin, Balázs Gulyás, Boglárka Makkai*, Péter Kása**, Jan Andersson, Kazatoshi Suzuki*** and Tetsuya Suhara***

*Karolinska Institutet, Stockholm, Sweden; *University of Debrecen, Hungary; **University of Szeged, Hungary; ***NIRS,Chiba, Japan*

During the past decades, in parallel with novel therapeutic approaches, the search for novel PET ligands of neuroinflammation and neurodegeneration has been intensified. In addition to the well known isoquinoline ligand PK11195, DAA1106 and vinpocetine have been labeled for both post mortem and in vivo imaging studies in our laboratory and the studied in detail in physiological and pathological conditions in both humans and primates.

In whole hemisphere human post mortem autoradiography studies, combined with immunohistochemical and immunoblot staining techniques, the cerebral uptake and binding pattern of 125I-labelled DAA1106 analogues were studied in both Alzheimer's disease (AD) and age matched control brains. The results were compared with similar studies performed in our laboratories, using labeled PK11195 and vinpocetine. DAA1106 has proved to be a useful marker of neuroinflammation in the brain parenchyma as validated by immunohistochemical stains. The brain uptake of 125-labelled DAA1106 analogues was 20-25 % higher in the hippocampus, parietal and temporal lobes in AD brain than in the corresponding regions in age matched control brains. DAA1106 binding could be effectively blocked by the two other PBR ligands, PK11195 and vinpocetine.

In human PET studies 11C-labelled PK11105 and vinpocetine were used as tracers in control subjects as well as AD, stroke and multiplex sclerosis (MS) patients. Similarly to the known age-dependent uptake differences, 11C-vinpocetine brain uptake increased linearly with age in control subjects. In stroke subjects, both PK11195 and vinpocetine could indicate the stroke region's boundaries, but the intensity of this effect varied widely, and it most probably depends upon the interval between the stroke and the imaging session. In MS patients, both ligands may be indicative of the lesion sites, but their sensitivity is low. In AD patients 11C-vinpocetine may prove to be an outstanding indicator of ongoing cerebral pathophysiological processes, as its regional uptake and binding potential is multifold in those brain regions affected by the disease as compared to healthy age matched controls. Improved PET tracers for neuroinflammation include 11C-DAA1106 and 18F-FEDAA1106. Preliminary PET results in human brain will also be discussed.

CARBON-11 AND FLUORINE-18 RADIOLIGANDS FOR IMAGING THE PERIPHERAL BENZODIAZEPINE RECEPTORS

F. Dollé*, M. Kassiou, F. Chauveau, N. Van Camp, C. Thominiaux, B. Kuhnast, H. Boutin, Ph. Hantraye and B. Tavitian

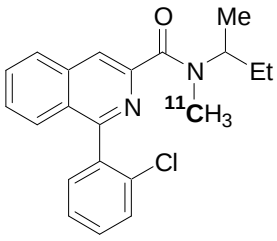
Service Hospitalier Frédéric Joliot, Institut d'Imagerie Biomédicale, CEA/DSV, 4 place du Général Leclerc, F-91401 Orsay, France.

**Tel: + 33 (0)1 69 86 77 04 ; Fax: + 33 (0)1 69 86 77 49 ; e-mail: frederic.dolle@cea.fr*

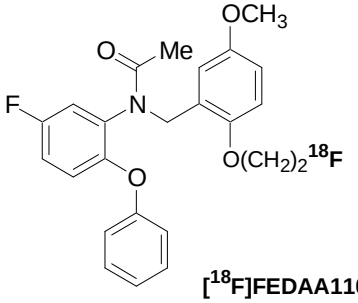
Peripheral benzodiazepine receptors (PBR or translocator protein (18 kDa, TSPO), a new name which has been recently coined [1]) are associated with the outer mitochondrial membrane in many tissues where they are modulated by hormones and drugs. Although the functional role of PBR in the CNS has not yet been fully clarified, it has been shown that they are implicated in the regulation of steroidogenesis, immunomodulation, cellular proliferation and apoptosis.

With the aim of finding a useful positron emission tomography (PET) imaging tool for the *in vivo* study of the PBR, several selective ligands have already been radiolabelled with the positron emitters carbon-11 (half-life : 20.38 minutes) and fluorine-18 (half-life : 109.8 minutes) [2,3]. These radioligands can be classified according to their structure into eight distinct groups [4]. The first group includes the 3-isoquinolinecarboxamide [^{11}C]PK11195 and some of its derivatives ([^{11}C]PK11211, [^{11}C]PK14105 and [^{18}F]PK14105). The second group is represented by the atypical benzodiazepine 4'-chlorodiazepam ([^{11}C]Ro5-4864) while the third group consists of a series of quinoline-2-carboxamides, structurally closely related to PK11195 ([^{11}C]VC193M, [^{11}C]VC198M, [^{11}C]VC195 and [^{11}C]VC701). The fourth group is made up of a series of *N*-benzyl-*N*-(2-phenoxyaryl)-acetamides, which includes [^{11}C]DAA1106, [^{18}F]FMDAA1106, [^{18}F]d_2FMDAA1106 and [^{18}F]FEDAA1106 as well as the recently reported pyridinylacetamide [^{11}C]PBR28. The fifth group is represented by [^{11}C]vinpocetine, a compound structurally related to the Vinca minor alkaloid vincamine. The sixth group consists of a series of 2-phenylpyrazolo[1,5-*a*]pyrimidineacetamides and includes [^{11}C]DPA-713. The seventh group consists of a series of 2-phenylimidazo[1,2-*a*]pyridine-acetamides and includes [^{11}C]CLINME. The eighth and last group is made up of a series of 2-aryl-8-oxodihydropurines which includes the recently reported [^{11}C]AC-5216.

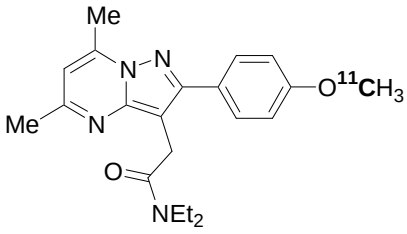
Among these radioligands, [^{11}C]PK11195 is not only the oldest, but also the most widely used tracer for imaging the PBR. However, this tracer suffers from low brain uptake and extensive binding to plasma proteins, which complicate a quantitative analysis of PBR density. Within the *N*-benzyl-*N*-(2-phenoxyphenyl)-acetamide series, [^{18}F]FEDAA1106 seems to be a promising alternative to the use of [^{11}C]PK11195, based on preliminary data obtained in humans. Exceptional *in vivo* binding properties have also been reported recently for the 2-phenylpyrazolo[1,5-*a*]pyrimidine-acetamide [^{11}C]DPA-713, which is currently being further evaluated in rodents and non-human primates [5-7].



[^{11}C]PK11195



[^{18}F]FEDAA1106



[^{11}C]DPA-713

References

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[2]. James ML et al., *Curr. Med. Chem.* 2006, 13: 1991-2001
[3]. Kassiou M et al., *Brain Research Reviews* 2005, 48: 207-210
[4]. Thominiaux C et al., *J. Label. Compounds Radiopharm.* 2007, in press
[5]. James ML et al., *Bioorg. Med. Chem.* 2005, 13: 6188-6194
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[7]. Boutin H et al., *J. Nucl. Med.*, 2007, in press.

PERIPHERAL BENZODIAZEPINE RECEPTOR IMAGING: [^{11}C]PK11195 CHALLENGERS

H. Boutin*, F. Chauveau, C. Thominiaux, M-C. Grégoire, M. L. James, R. Trebossen, Ph. Hantraye, F. Dollé, A. Katsifis, M. Kassiou, B. Tavitian.

*Faculty of Life Sciences, Michael Smith Building, University of Manchester, Oxford road, Manchester M13 9PT – UK - Tel.: +44 (0) 161 275 5039 - Fax: +44 (0) 161 275 5948 - email: herve.boutin@manchester.ac.uk

Microglial cells are important contributors to the immune response in the brain. They are activated in several types of acute or chronic neuropathological conditions such as brain trauma, stroke or Alzheimer's and Parkinson's diseases. Microglial cells when activated express peripheral benzodiazepine receptors that have been used for several years as a biomarker of neuroinflammatory processes, mainly through the use of [^{11}C]PK11195 in PET imaging. However, as [^{11}C]PK11195 characteristics highlight weaknesses mainly in terms of modelling and therefore quantification of the data, great efforts have been put into development and characterization of numerous novel candidates for PBR PET imaging.

Although several new ligands have been developed and tested, most of the results provided so far in the literature are from *ex vivo* or *in vitro* studies. Indeed, Maeda *et al.* [1], Zhang *et al.* [2,3] and Fujimara *et al.* [4] have extensively described [^{11}C]DAA1106 and fluorinated derivatives in *ex vivo* binding experiments on rat brain sections and biodistribution in mice. They also performed PET imaging in normal monkeys or healthy patients, but so far no published data from *in vivo* experiments combining DAA1106 and derivatives in brain with enhanced microglial activation have been published. Guylas *et al.* [5,6] have investigated [^{11}C]vinpocetine using PET but only in healthy patients or monkeys, and pharmacological data indicate that vinpocetine may bind to other binding sites than the PBR [6]. Belloli *et al.* [7] have evaluated carbon-11 labelled VC193M, VC195 and VC198M in a rat model of striatal lesion (quinolinic acid), and their *in vivo* distribution by microdissection of the brain. These authors have shown that VC195 depicted *in vivo* biodistribution and binding similar to PK11195 and therefore had the same potential as PK11195 as a PBR ligand. We have recently performed evaluation of [^{11}C]DPA-713 [8] and [^{11}C]CLINME [9], 2 new PBR ligands, using *in vivo* microPET imaging in rodents, and show great potential for these 2 compounds with an enhanced signal to noise ratio when compared to [^{11}C]PK11195 microPET images.

Overall, this highlights the need for an associated effort to standardise the evaluation of these various compounds using different experimental models (e.g. stroke, excitotoxic brain lesion, transgenic model of neurodegenerative diseases) to evaluate their *in vivo* binding characteristics, and validate their quantification and modelling to be able to bring the best candidate to clinical imaging.

References:

[1]. Maeda J. *et al.* (2004) *Synapse* 52:283-291
[2]. Zhang M.R. *et al.* (2003) *Nucl.Med.Biol.* 30:513-519
[3]. Zhang M.R. *et al.* (2004) *J.Med.Chem.* 47:2228-2235
[4]. Fujimura Y. *et al.* (2006) *J Nucl.Med.* 47:43-50
[5]. Gulyas B. *et al.* (2005) *J.Neurol.Sci.* 229-230:219-223
[6]. Gulyas B. *et al.* (2002) *Acta Neurol.Scand.* 106:325-332
[7]. Belloli S. *et al.* (2004) *Neurochem.Int.* 44:433-440
[8]. Boutin H. *et al.* (2007) *J Nucl.Med.* (in press)
[9]. Boutin H. *et al.* (2007) *Glia* (submitted).

PET IMAGING OF PBR IN A RAT MODEL OF HSV ENCEPHALITIS

J. Doorduin[1], H.C. Klein[1], M. James[2], M. Kassiou[2], R.A. Dierckx[1], E.F.J. de Vries[1]

1, Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands; 2, Brain and Mind Research Institute, University of Sydney, Campertown, Australia, NSW 2050

Phone 0031-50-3613909; Fax 0031-50-3611687; e-mail: j.doorduin@pet.umcg.nl

Neuroinflammation is associated with a variety of neurological diseases. During neuroinflammation the expression of the peripheral benzodiazepine receptor (PBR) is increased, which can be visualised by positron emission tomography (PET) with [^{11}C]PK11195. However, [^{11}C]PK11195 shows low brain uptake and high non-specific binding and may not be sensitive enough to visualise mild inflammation. Recently, [^{11}C]DPA-713 and [^{18}F]DPA-714 were developed as potentially more sensitive PET tracers for PBR than [^{11}C]PK11195.

Both [^{11}C]DPA-713 and [^{18}F]DPA-714 were evaluated in a rat model of herpes simplex encephalitis (HSE) and compared to [^{11}C]PK11195.

The uptake of both [^{18}F]DPA-714 and [^{11}C]PK11195 was significantly higher in affected brain areas in HSE rats as compared to the corresponding brain areas in controls. [^{11}C]DPA-713 uptake was not significantly increased. In controls, basal uptake of [^{18}F]DPA-714 was significantly lower than [^{11}C]PK11195 uptake.

Thus, [^{18}F]DPA-714 is a promising new tracer for neuroinflammation, especially because of its better contrast between inflamed and non-inflamed areas.

QUANTIFYING [11C]PK11195 UPTAKE IN HIV DEMENTIA

Endres Christopher and Martin Pomper

Johns Hopkins University Radiology, Baltimore USA

Central nervous system (CNS) infection with human immunodeficiency virus (HIV) is associated with the development of neurocognitive decline involving motor function (e.g., slowed movements, abnormal gait, hypertonia), behavior (e.g., apathy, irritability, emotional lability), and cognition (e.g., attention, concentration, memory, information processing, language). A severe form of neurocognitive decline is HIV-associated dementia (HAD), whereas a milder form is referred to as minor cognitive motor disorder or MCMD. The primary difference between HAD and MCMD is the degree of impairment in daily function. As to the etiology of impairment, neurons are not believed to be infected actively by HIV. Rather, it is believed that the majority of neurological damage in HAD is the result of glial cell activation by HIV-infected monocytes, inducing the release of viral neurotoxins as well as a multitude of cytokines and chemokines, which contribute to neuronal damage and apoptosis. The resulting neuronal cell damage from these compounds leads to the activation of glial cells. In their active state, glial cells show increased expression of the peripheral benzodiazepine receptor (PBR), consequently, increased PBR binding is expected in HAD. In a study using the PBR ligand [^{11}C]-*R*-PK11195 with PET, we have examined PBR binding in HAD (n=5), HIV subjects with no dementia (HIV-ND, n=5), and controls (n=5). It was determined that HAD patients showed significantly higher [^{11}C]-*R*-PK11195 binding than controls in five out of eight brain regions ($P < 0.05$, Mann Whitney U test). HIV-ND patients did not show significantly increased binding compared to controls, and HIV-ND patients also did not show significantly different binding from HAD patients. Although discrimination of PBR binding in the three subject groups was not proven statistically, these data indicate that there is a rank order of PBR binding of HAD > HIV-ND > controls as is expected. These results support a role for glial cell activation in HAD, and that PET with [^{11}C]-*R*-PK11195 can detect the concomitants of neuronal damage in individuals infected with HIV. However, despite these promising preliminary results, [^{11}C]-*R*-PK11195 suffers from substantial nonspecific binding and difficulty in quantification. Accordingly others and we have begun to pursue other PBR markers as will be discussed.

NEW TRACERS FOR TRANSLOCATOR PROTEIN (18 KDA) (TSPO) EXPLORATION BY SPECT AND PET: PRECLINICAL STUDIES

S. Chalon[1], A. Katsifis[2], M. James[3], N. Arlicot[1], F. Mattner[2], R. Banati[3], S. Meikle[3], J. Vercouillie[1], D. Guilloteau[1], M. Kassiou[3]

[1]Inserm U619, Tours, France; [2]ANSTO, Sydney, Australia; [3]University of Sydney, Australia

The translocator protein (TSPO), previously known as the peripheral-type benzodiazepine receptor (PBR) is minimally expressed in normal brain parenchyma, where it is primarily localized in glial cells. Its basal expression raises in several neurodegenerative disorders, due to the presence of infiltrating inflammatory cells and activated microglia. Accordingly, TSPO is a potential target to evaluate by molecular imaging in vivo neuroinflammatory changes in a variety of neurological diseases. For this aim several SPECT and PET tracers are currently developed.

We propose to validate potential in vivo properties of these tracers in pathological situations associated to brain injury using a know rat model of striatal excitotoxic lesion. This lesion is induced in male Wistar rats by intrastriatal injection of different amounts of quinolinic acid (QA) (75, 150 or 300 nmol), in order to simulate different clinical stages severity.

We performed ex vivo cerebral biodistribution studies with different TSPO tracers in this animal model and compared the labelling obtained with these compounds to the signal obtained using a complementary immunohistochemistry method with the marker of activated microglia OX-42. Two new molecular imaging compounds were tested to date, i.e. CLINDE (K_i=1.7 nM) potentially useful for SPECT and DPA-714 (Ki=7 nM) for PET.

One week after stereotaxic brain lesion, animals received an i.v. injection of either [^{125}I]CLINDE or [^{18}F]DPA-714 with or without a pre-injection with the reference TSPO compound PK11195 (5mg/kg). The brain was removed either 30 or 60 min after tracer injection, then the radioactivity and weight of several brain areas were measured. Ex vivo autoradiography and immunohistochemical studies were performed on coronal brain sections in other animals submitted to similar lesions.

We observed for both ligands a higher striatum/cerebellum ratio in the lesioned than in intact side, i.e. around 3 for CLINDE and 8 for DPA-714 after a lesion with 300 nM QA. This striatal accumulation in the lesioned side was in all cases abolished by a pre-injection of PK-11195, thus indicating the in vivo specificity of tracers binding to TSPO.

Ex vivo autoradiography and immunohistochemistry studies on brain sections were consistent with these results, revealing a good spatial correspondence between radioactivity signal and neuroinflammatory foci. Moreover, regression analysis yielded a significant correlation between the amount of CLINDE binding and the clinical severity of QA lesion (r^2 = 0.9967 and r^2 = 0.9994 in ipsilateral striatum and cortex, respectively, $p<0.001$).

These preliminary results demonstrate that both tracers are potentially useful for TSPO in vivo imaging in neurodegenerative disorders associated with microglial activation.

QUANTITATIVE ANALYSIS OF [C-11]-(*R*)-PK11195 PET BRAIN STUDIES.

Boellaard, B.N.M. van Berckel, M.A. Kropholler, A.S. Schuitemaker, P. Scheltens, A.A. Lammertsma.

VU University Medical Centre, Dep.of Nuclear Medicine & PET Research, P.O.Box 7057,1007 MB Amsterdam, The Netherlands.

Phone 0031-20-4442857; Fax –31-20-4443090; E-mail r.boellaard@vumc.nl

(*R*)-[^{11}C]PK11195 may be used for quantifying cerebral microglial activation *in vivo*. In this abstract an overview of work performed in our institute on the evaluation of quantitative methods for analysing (*R*)-[^{11}C]PK11195 PET brain studies in MCI and AD is given. First, plasma input and reference tissue models were evaluated and it was shown that a reversible two tissue compartment model with K_1/k_2 fixed to whole cortex was the optimal model when a metabolite corrected arterial plasma input function was available, whilst the simplified reference tissue model proved to be best in absence of plasma input. Next, various anatomical regions and cluster analysis for definition of reference regions were compared and we found that total matter cerebellum provided best trade-off between fit accuracy and discrimination of binding between subject groups. Comparison of various parametric methods revealed that reference parametric mapping (RPM) was able to provide quantitative accurate parametric binding potential (BP) images. SPM analysis using these RPM data showed increased binding in elderly and AD subjects in several regions, which were not detected using ROI based analysis. Finally, application of PVC generally resulted in increased BP at the cost of reduced precision and did not improve discrimination of PK11195 binding between subjects groups.

PERFORMANCE EVALUATION OF VARIOUS PARAMETRIC METHODS FOR [^{11}C]PIB

M. Yaqub, B.N.M. van Berckel, N. Tolboom, B. Windhorst, G. Luurtsema, P. Scheltens, A.A. Lammertsma, R.Boellaard.

VU University Medical Centre, Dep.of Nuclear Medicine & PET Research, P.O.Box 7057,1007 MB Amsterdam, The Netherlands.

Phone 0031-20-4442857; Fax –31-20-4443090; E-mail r.boellaard@vumc.nl

[^{11}C]PIB is a ligand for imaging amyloid load in brain. The aim of the present study was to investigate performance of various parametric methods including those in which the reference tissue efflux rate (k_2') is fixed to improve signal to noise ratio. The following methods were studied: RPM1, Reference Logan, various multi-linear methods ($MRTM_{0-2}$) and RPM2 with fixed k_2'. Both simulations and clinical data were used to assess precision and accuracy of parametric BP and DVR under various conditions. In addition, test-retest studies were performed in 6 control and 5 AD subjects. For clinical data all methods showed good correlation with SRTM (R^2=0.89-95). Best correlation was seen for RPM1. Difference in average BP between AD and control subjects was (P<0.001), however, slightly smaller for RPM1 and RPM2 than for the other methods. Finally, DVR test-retest variability was better than 4.1% for all methods. Simulations and clinical data indicated that MRTM, RPM1, RPM2 and MRTM2 were most accurate parametric methods for [^{11}C]PIB studies. Fixing k_2' had a beneficial effect on the performance of these methods. Visual inspection of parametric images also revealed a small improvement of image quality using k_2' fixed. RPM2 is recommended for parametric analysis of [^{11}C]PIB studies.

EVALUATION OF COMPARTMENT MODELS AND SEMI-QUANTITATIVE MEASURES FOR ANALYSIS OF ^{18}F-FDDNP PET STUDIES.

M. Yaqub, R.Boellaard, B.N.M. van Berckel, N. Tolboom, B. Windhorst, G. Luurtsema, P. Scheltens, A.A. Lammertsma.

VU University Medical Centre, Dep.of Nuclear Medicine & PET Research, P.O.Box 7057,1007 MB Amsterdam, The Netherlands.

Phone 0031-20-4442857; Fax –31-20-4443090; E-mail r.boellaard@vumc.nl

^{18}F-FDDNP is a PET ligand for imaging of beta-amyloid in human brain. This study evaluates quantification of ^{18}F-FDDNP PET studies using pharmacokinetic compartment models and semi-quantitative measures. Different kinetic and semi-quantitative methods were assessed: plasma input single tissue (1T-2k), two tissue irreversible (2T-3k) and reversible (2T-4k) compartment models, simplified (SRTM) and full (FRTM) reference tissue models, standard uptake values (SUV) and SUV ratios (SUVr). Both simulations and clinical evaluations were performed. Dynamic PET studies were performed with 4 control and 3 AD subjects.

AIC indicate a preference for 2T-4k in case of plasma input and SRTM for reference input. Reference tissue models may provide more stable results as plasma input function data are hampered by very rapid metabolism of the tracer. Evaluation of 2T-4k parameters revealed a possible increase of k4 and decrease of BP for AD versus healthy subjects, possibly due to the contribution of metabolites. BP-SRTM was higher for AD (0.089 versus 0.075). More clinical ^{18}F-FDDNP evaluations are underway to further assess use of plasma input models and to validate SRTM and simplified methods (e.g. SUVr and DVR).

EVALUATION OF COMPARTMENT MODELS AND SEMI-QUANTITATIVE MEASURES FOR ANALYSIS OF ^{18}F-FDDNP PET STUDIES.

M. Yaqub, B.N.M. van Berckel, N. Tolboom, B. Windhorst, G. Luurtsema, P. Scheltens, A.A. Lammertsma, R.Boellaard.

VU University Medical Centre, Dep.of Nuclear Medicine & PET Research, P.O.Box 7057,1007 MB Amsterdam, The Netherlands.

Phone 0031-20-4442857; Fax –31-20-4443090; E-mail r.boellaard@vumc.nl

^{18}F-FDDNP is a PET ligand for imaging of beta-amyloid in human brain. The purpose of this study is to determine performance of several reference tissue parametric methods for assessing [^{18}F]FDDNP binding. Various parametric methods were evaluated: reference Logan, two basis function methods (RPM1 & RPM2), and various multi-linear methods (MRTMo, MRTM, MRTM2). RPM2 and MRTM2 all include fixing the reference tissue clearance rate (k_2'). Simulations and clinical dynamic PET studies (3 controls, 3 MCI, 3 AD) were used to determine accuracy and precision of parametric BP. For most methods, clinical parametric BP data showed correlations of R^2=~0.8 with BP-SRTM. The only exception was MRTM (R^2=0.49). Lowest biases were found for MRTM2 and RPM2 (1 and 3%, respectively). Simulations confirmed results observed in clinical data. RPM2 parametric data showed a trend for increased BP in AD subjects in line with findings from SRTM. Based on observed accuracies and precisions and image quality RPM2 is recommended for generating parametric BP images. Another advantage of RPM2 is that is provides parametric data of the regional flow distribution (R1).

[^{11}C]PK11195 AS A MARKER OF INFLAMMATION IN A MODEL OF STROKE

Santiago Rojas[1], Abraham Martín[1], Maria J. Arranz[1], Deborah Pareto[2], Jesus Purroy[1], Esther Verdaguer[1], Jordi Llop[2], Vanessa Gómez[2], Joan D. Gispert[2], Olga Millán[2], Ángel Chamorro[3], and Anna M. Planas[1]

1 Department of Pharmacology and Toxicology, Institut d'Investigacions Biomèdiques de Barcelona (IIBB)-Consejo Superior de Investigaciones Científicas (CSIC), Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; 2 Institut d'Alta Tecnología (IAT), Barcelona, Spain; 3 Stroke Unit, Institut Clínic de Malalties del Sistema Nerviós (ICMSN), Hospital Clínic, IDIBAPS; Barcelona, Spain

[^{11}C]PK11195 is used in PET studies for imaging brain inflammation *in vivo* as it binds to the peripheral-type benzodiazepine receptor (PBR) expressed by reactive glia and macrophages. However, the features of the cellular reaction required to induce a positive signal with [^{11}C]PK11195 are not well characterized. We performed [^{11}C]PK11195 PET studies in rats following transient focal cerebral ischemia. We determined [^{3}H]PK11195 binding and PBR expression in brain tissue and examined the lesion with several markers. The standard uptake value of [^{11}C]PK11195 increased at day 4, grew further at day 7 within the ischemic core, but augmented to a lesser extent in surrounding regions. Accordingly, *ex vivo* [^{3}H]PK11195 binding increased at day 4, and rose further at day 7. We also found parallel increases in the expression of PBR mRNA, and we detected a 18-kDa band corresponding to PBR protein by Western blotting in the ipsilateral hemisphere. Immunohistochemistry against PBR showed subsets of ameboid microglia/macrophages within the core of infarction with a distinctive strong PBR expression from day 4. These cells were often located surrounding zones of microhemorrhages. Reactive astrocytes showing positive PBR immunostaining were occasionally found in peripheral regions. These results show cellular heterogeneity in the level of PBR expression, supporting that PBR is not a simple marker of inflammation, and that the extent of [^{11}C]PK11195 binding depends on intrinsic features of the inflammatory cells.

PET AND MOLECULAR IMAGING IN PRECLINICAL MODELS OF INFLAMMATION AND NEURODEGENERATION

S. Belloli, R.M Moresco and F. Fazio.

Institute of Molecular Bioimaging and Physiology-CNR, University of Milano-Bicocca, San Raffaele Institute, Via Olgettina 60, 20132 Milan, Italy.

Phone 0039 02 26433817; Fax 0039 02 26415202 ; E-mail moresco.rosamaria@hsr.it; belloli.sara@hsr.it

Molecular imaging techniques are powerful instruments to detect and monitor neuroinflammatory and neurodegenerative processes. In particular Positron Emission Tomography allows the *in vivo* measurement of different biochemical pathways in living subjects. The development of dedicated tomograph has allowed the application of PET molecular imaging to the longitudinal assessment of preclinical models of disease including Huntington (HD) and Parkinson disease (PD). Using these techniques we have evaluated *in vivo* and validated *in vitro* an excitotoxic model of HD based on the monolateral striatal injection of the neurotoxin quinolinic acid (QA). The rate of progressive loss of intrastriatal neurons and the presence of inflammatory reactions such as microglia activation have been monitored up to two months after QA administration by *in vivo* and *ex-vivo* PET techniques. In particular the expression of three different biochemical markers linked to striatal neurons or inflammation: the D_2 dopamine receptor, the A_{2A} adenosine receptor and the peripheral benzodiazepine receptor (PBR) has been evaluated using [^{11}C]Raclopride, [^{11}C]SCH442416 and [^{11}C]PK11195 respectively.

The results of this study together with limits and potentiality of preclinical PET imaging will be summarized and presented.

IMAGING NEURONAL AND MICROGLIAL ACTIVATION DURING SEIZURES IN GENETICALLY MODIFIED MICE

Martine M. Mirrione[1,2], Wynne K. Schiffer[2], Joanna S. Fowler[2], Dave L. Alexoff[2] Stephen L. Dewey[1,2,3], Stella E. Tsirka[1]

1Graduate Program in Molecular and Cellular Pharmacology, State University of New York at Stony Brook, Stony Brook, New York 11794; 2Medical Department, Brookhaven National Laboratory, Upton, New York 11973 ; 3Psychiatry Department, New York University, School of Medicine, New York, New York 10016

Increased hippocampal excitability is commonly observed in temporal lobe epilepsy (TLE) and can be visualized indirectly using metabolic imaging of 2-deoxy-2[18F]fluoro-D-glucose (^{18}FDG). Medically-refractory seizures cause inflammation and neurodegeneration and seizure initiation thresholds have been linked in mice to the serine protease tissue plasminogen activator (tPA). Previous research has shown that mice lacking tPA exhibit resistance to seizure induction, and the ensuing inflammation and neurodegeneration are similarly suppressed. Employing statistical parametric mapping (SPM) methods for small animal PET data analysis, we examine patterns of ^{18}FDG uptake in genetically modified mice and find that they correlate with the severity of drug-induced seizure initiation. Furthermore, we consider the role of microlgial activation during seizures.

VISUALIZATION OF MICRO- AND ASTROGLIA IN HEALTH AND DISEASE STATES BY TWO-PHOTON LASER SCANNING MICROSCOPY,

Frank Kirchhoff

Neurogenetics, Max Planck Institute of Experimental Medicine, Göttingen, Germany

Phone 0049 551 3899 770; Fax 0049 551 3899 758; E-mail Kirchhoff@em.mpg.de

Current progress in our understanding of neurological diseases is tremendously facilitated by the development of new transgenic mouse models, as well as by advanced electrophysiological and imaging techniques. Transgenic mice expressing various fluorescent proteins (FPs) under the control of neural promoters are are perfect tools to study cellular interactions *in vivo*. We generated and used such mice to study the neuron-glia interactions and immediate cellular responses upon acute injuries in cortex or spinal cord by *in vivo*-two photon laser-scanning microscopy. After anaesthesia mice could be maintained for up to 11 h. To study spinal cord injuries, a laminectomy was performed at spinal cord segment L4. To minimize movements in the region of interest, the adjacent spines were rigidly fixed. Neurons, astrocytes or microglia could be imaged at high spatial resolution. In the resting CNS tissue microglial processes displayed a high degree of motility and moving along axonal tracts surveying the cellular environment. Upon minimal laser lesioning of axons within the upper layers of the dorsal spinal cord, microglial cells sent out their processes immediately and enwrapped degenerating axonal compartments.

This study demonstrates the potential of *in vivo*-imaging to uncover the sequence of immediate cellular responses during traumatic injuries within the central nervous system.

A STUDY OF IMMUNOLOGICAL RESPONSE TO BRAIN ISCHEMIA USING FMT

J. Aguirre, A. Muñoz, A. Garofalakis, R. Favicchio, H. Meyer, G. Zacharakis, E.N. Economou, C. Mamalaki(2),D. Kioussis(3), and J. Ripoll .

IESL – FORTH, (2) IMBB – FORTH, (3)NIMR-F. FORTH, VASILIKA BOUTON PO BOX 1385, 7110 HERAKLION, CRETE, GREECE

Phone 00302810391109; Fax 00302810391101; E-mail juanaguir@gmail.com

Given the fact that Optical Tomography in tissues is capable of quantitatively imaging the distribution of several important chromophores and fluorophores in-vivo, there has been great interest in developing optical imaging systems with increased number of measurements under optimal experimental conditions.

A suitable technique for in-vivo optical imaging is Fluorescence Molecular Tomography, a novel imaging system that enables three dimensional (3D) imaging of fluorescent probes in whole animals in a non-contact geometry, in combination with a 3D surface reconstruction algorithm.

Some studies suggest that within some days after ischemia, immunodepression might occur. Our study has been quantitatively imaging the spleen, the thymus and the ganglia of C-57 Bl/10 transgenic mice which all its T-cells express GFP protein, 1 day, 2 days, 4 days and 7 seven days after causing ischemia, by sectioning the middle cerebral artery . After each FMT measurements a blood FACs analysis was performed, and after the last FMT measurement FACs analysis was performed with the organs it selves, in order to compare them with the FMT results. Any trend in the immunological response shall be presented. Having combined FACS and FMT will also allow us to confirm the potential of FMT for quantitatively imaging GFP expressing T-cells in living mice.

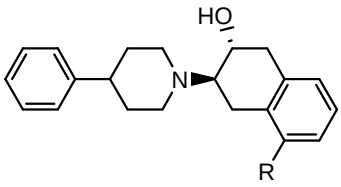
Keywords: Non-contact imaging, fluorescence molecular tomography, whole-animal imaging, gene expression, optical tomography, fluorescence.

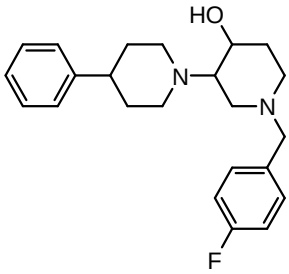
IS THE VESICULAR ACETYLCHOLINE TRANSPORTER (VACHT) A GOOD TARGET FOR NEURODEGENERATIVE DISEASE?

M. Kassiou1, M. Allard2, J. Mazère2, N. Giboureau3, J. Vergotte3, P. Emond3, D. Guilloteau3

[1]Brain and Mind Research Institute, University of Sydney, Australia; [2] Service de médecine nucléaire, CHU de Bordeaux1, [3]INSERM U619, Université François Rabelais de Tours, France

The vesicular acetylcholine transporter (VAChT) is a glycoprotein responsible for the accumulation of acetylcholine (ACh) into synaptic vesicles and is located exclusively on presynaptic cholinergic neurons. The interest in targeting the VAChT in neurodegeneration and in particular Alzheimer's disease (AD) is based upon the observation that as disease progresses, levels of the VAChT change in parallel fashion and magnitude with other cholinergic markers, particularly choline acetyltransferase (ChAT) [1]. This suggests that molecular imaging of the VAChT may provide insights into early diagnosis and a better understanding of AD and its progression. To achieve this goal, several radioligands have been developed with the most widely used to date being [123I]IBVM. Results of a preliminary SPECT clinical study showed a significant decrease of [123I]IBVM binding in AD compared to controls. Moreover, in these patients, [123I]IBVM binding was negatively correlated with the disease progression.





- R = I IBVM FBT
- R = N(Et)COCH2F NEFA
- R = O(CH2)n-X

n=2 ; X= F FEOBV

n=3 ; X= F FPOBV

Several [l8F]-labelled VAChT radioligands have also been synthesised including [18F]NEFA, [18F]FBT, and [18F]FEOBV. Both [18F]NEFA and [18F]FBT displayed high affinity for the VAChT (Ki = 4.4 nM and 0.44 nM respectively). Evaluation of [18F]NEFA in Cynomolgus monkey resulted in a heterogeneous distribution that mirrors the density of cholinergic terminals in the brain. Nevertheless human PET studies demonstrated no binding in the cortex, a region known to contain the VAChT. A number of PET studies have been performed with [18F]FBT, although [18F]FBT was never been used in any human study. In rodents [18F]FEOBV has demonstrated a biodistribution consistent with the known density of the VAChT. However, no competitive blocking studies have been performed in mice or rats. Therefore the specificity of this radioligand has only been assumed based on its distribution pattern. To date no human PET studies have been reported using [18F]FEOBV. This presentation will review recent advances in the development of radioligands for imaging the VAChT including the further characterisation of [18F]FEOBV and the evaluation of the fluoropropoxy analogue 5-FPOBV. This new analogue of FEOBV, which we recently described displayed high affinity in vitro for the VAChT (KD=0.77 nM) [2] and could afford a radioligand with a different pharmacokinetic profile.

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BASIC ASPECTS OF INFLAMMATION IN CNS BACTERIAL INFECTIONS AND POTENTIAL TARGET CANDIDATES FOR IMAGING

Tammy Kielian,

Ph.D. Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, AR 72205 USA

Brain abscesses form in response to a parenchymal infection with pyogenic bacteria, with *S. aureus* representing a common etiologic agent of human disease. Brain abscesses are typified by extensive edema and tissue necrosis and tend to localize at white-grey matter junctions where microcirculatory flow is poor. In addition to the sequential progression from cerebritis to necrosis during brain abscess evolution, the activation of resident glial cells and influx of peripheral leukocytes demonstrate temporal patterns. Based upon its prevalence in human CNS infections, our laboratory has utilized *S. aureus* to establish an experimental brain abscess model in the mouse that accurately reflects the course of disease progression in humans, providing an excellent model system to identify critical molecules responsible for the establishment of CNS anti-bacterial immunity.

Identifying the signals, effectors, and sequence of innate immune responses in the experimental brain abscess model has been a focus of our laboratory over recent years. Of particular interest has been defining the potential impact of proinflammatory cytokines, chemokines, and pattern recognition receptors on the disease process and how these processes may be regulated with pharmacological interventions to improve brain abscess sequalae. From these studies, we have identified several molecules that play a key role in regulating the host immune response to bacterial infection in the brain, some which are beneficial and others we believe to be detrimental if not tightly regulated.

The focus of this presentation will be to discuss the chain reactions that are elicited during neuroinflammation in brain abscesses and thoughts about which of these processes may be important to track with future neuroimaging modalities.

RADIOLABELED COX-2 INHIBITORS AS PROBES FOR PET IMAGING

E. F. J. de Vries, J. Doorduin, A. van Waarde, R. A. Dierckx

University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, the Netherlands.

Phone: +31-50-3613599; Fax: +31-503611687 ; E-mail: e.f.j.de.vries@pet.umcg.nl

Cyclooxygenase-2 (COX-2), a key enzyme in the biosynthesis of prostaglandins, plays an important role in neuroinflammation and neuroprotection. COX-2 is overexpressed in affected brain areas in many neurodegenerative diseases. In several neurodegenerative diseases, COX-2 inhibitors were found to have a prophylactic effect.

We have labeled the selective COX-2 inhibitors Celecoxib (CEL) and Rofecoxib (ROF) with carbon-11 and evaluated their utility as PET tracers for COX-2 in healthy rats and in a rat model of herpes simplex encephalitis (HSE) using microPET and ex vivo biodistribution.

In healthy rats, brain uptake of [^{11}C]CEL (SUV 0.9–1.1) was higher than that of [^{11}C]ROF (SUV 0.2–0.4). However, [^{11}C]CEL brain uptake was homogeneous, whereas [^{11}C]ROF distribution did correspond to the known COX-2 distribution. Upon treatment with the COX-2 inhibitor NS398, [^{11}C]ROF uptake was significantly reduced in some brain regions. In HSE rats, [^{11}C]ROF brain uptake was 5–58% higher than in healthy controls, but this increase was not statistically significant. [^{11}C]ROF uptake did not correspond to microglia activation, as determined by [^{11}C]PK11195 PET.

Thus, [^{11}C]CEL and [^{11}C]ROF appear not sensitive enough to monitor COX-2 expression with PET, probably due to high non-specific binding.

PHENOTYPING OF MOUSE MODELS OF ALZHEIMER'S DISEASE BY PET

[1,2]A. Winkeler, [3]N. Galldiks, [1,3]Y. Waerzeggers, [1,2,3]A.H. Jacobs.

[1]Laboratory for Gene Therapy and Molecular Imaging at the Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max-Planck Society and the Faculty of Medicine of the University of Cologne, [2]Center for Molecular Medicine and [3]Department of Neurology at the University of Cologne, Cologne, Germany,

Objective: To characterize newly developed and existing mouse models of neurodegenerative diseases (ND) *in vivo* by positron emission tomography.

Background: Non-invasive imaging by positron emission tomography (PET) has been shown to be a useful tool in diagnostics of patients with neurodegenerative diseases. Alzheimer's disease (AD) patients e.g. show a characteristic pattern of altered cerebral glucose metabolism, flumazenil binding and acetycholine esterase activity.

Mouse models of Alzheimer's diseases and newly developed mouse models with direct interest in this topic may be of specific value for the analysis, understanding and eventually therapy of this disease. We therefore believe that non-invasive imaging of mouse models of neurodegenerative disease will help us to characterize and phenotype these models in more detail and may be of great interest in monitoring early events, disease progression and potential treatment strategies.

Methods: Different mouse models of AD or mouse models investigating cross-connections to neurodegenerative diseases have been monitored by multi-tracer PET, with special focus on ^{18}F-FDG-PET. APP23 mice with or without locus ceruleus degeneration have been monitored to check for similar changes as AD patients, therefore the steady state of [^{18}F]FDG uptake, [^{11}C]FMZ binding and [^{11}C]MP4A trapping has been assessed by microPET *in vivo*. Furthermore, in the context of a potential molecular link between diabetes and neurodegenerative disease, we have performed FDG-PET imaging of brain/neuron-specific insulin receptor knockout (NIRKO) as well as insulin receptor substrate 2 knockout (IRS2-/-) mice crossed with APP2576 mice. In collaboration PET imaging results have been compared with the molecular analysis of mouse brain as well as behavioural analysis.

Results: The different mouse models do not show significant differences in [^{18}F]FDG brain-to-background ratios neither between control and APP23, control and NIRKO nor control and IRS-/- mice. Although not significant, IRS-/- crossed with APP2576 mice demonstrate reduced [^{18}F]FDG uptake in the brain. Only APP23 mice with Locus Ceruleus (LC) degeneration leading to noradrenergic depletion show a significant alteration of cerebral glucose metabolism, neuronal integrity as well as acetylcholine esterase activity as assessed by multi-tracer microPET *in vivo*.

Conclusions: Non-invasive imaging via PET seems to be a useful tool to characterize and phenotype animal models of neurodegenerative disease .

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PHENOTYPING OF MOUSE MODELS OF ALZHEIMER'S DISEASE BY MRI: ANATOMY, AMYLOID AND FUNCTIONAL IMAGING.

El Tannir El Tayara Nadine1, Volk Andreas1, Kober Frank2, Christine Walczak1, Delatour Benoît3, Dhenain Marc1,4.

[1]Institut Curie/INSERM U759, France, [2]CRMBM, UMR CNRS 6612, France [3]NAMC, CNRS, UMR 8620, France, [4]CEA-CNRS URA 2210, France

Phone +33 1 69 86 77 58; Fax + 33 1 69 86 77 45 E-mail Marc.Dhenain@cea.fr; Nadine.El-Tayara@curie.u-psud.fr

Alzheimer's disease (AD) is a major dementia that is characterized by severe brain atrophy and by its microscopic lesions that are amyloid plaques and neurofibrilary tangles. APP/PS1 mouse models of amyloid pathology have been developed to evaluate the effect of amyloid on the brain and the efficiency of new amyloid lowering drugs. During our studies, we used MRI to characterize the brain of these mice.

First, age related cerebral atrophy was evaluated. Despite a severe amyloid load in the cortex and hippocampus, the APP/PS1 mice did not present with cortical/hippocampal atrophy. On the other hand, they displayed an atrophy of white matter tracts.

Second, MRI protocols were developed to detect amyloid plaques. In a first attempt, direct MR imaging of amyloid plaques was performed by using a protocol called passive staining during which brains were soaked in a gadoteric acid solution (Dotarem®, Guerbet-France). In vivo imaging was also performed and plaques from the thalamus were detected on T2*-weighted MR images. Plaque detection in the thalamus was caused by the presence of iron positive aggregates within amyloid deposits. In vivo detection of the amyloid load was also possible by evaluating transversal relaxation times (T2) that were correlated to the amyloid load.

Third, the effect of amyloid on the cerebrovasculature was characterized by magnetic resonance angiography (MRA). Vascular alterations were mainly detected on the middle cerebral artery (MCA) from APP/PS1 mice and an age-related alteration of the MCA was detected in these mice.

Fourth, intracerebral perfusion was assessed, under normo- and hypercapnia conditions, in APP/PS1 mice and also in a more aggressive model of amyloidosis (APP/PS1KI mice). APP/PS1 and APP/PS1KI animals both showed a significantly reduced cerebral blood flow in the parietal cortex as compared to control mice.

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ANALYSIS OF NEURODEGENERATIVE AND NEUROINFLAMMATORY CHANGES UPON LOCUS CEURLEUS CELL DEATH IN TRANSGENIC MOUSE MODESL OF ALZHEIMER DISEASE.

Michael T. Heneka, Markus Kummer, Lars Tatenhorst, Stephan Paxian, Thea Hammerschmidt, Lucia Dumitrescu-Ozimek, Fred Van Leuven, Douglas L. Feinstein, Andreas H. Jacobs, Matthias Staufenbiel.

Dept. of Neurology, Molecular Neurology, University Münster, Münster, Germany.

The locus ceruleus (LC) is the main site of cerebral norepinephrine (NE) production. Profound degeneration of LC subregions which predominantly project to the neocortex and hippocampus occurr early in Alzheimer's disease (AD). Subsequently, cortical and hippocampal NE levels are decreasing. Since NE not only acts as a classical neurotransmitter but also modulates neuroinflammation, we evaluated NE effects on microglial functions including cytokine generation, migration and phagocytosis *in vitro* and *in vivo*. Using primary murine microglial cells and APPV717I transgenic mice we found that NE stimulation (10µM) abolished amyloid beta (Aβ) stimulated microglia chemokine and cytokine expression, while the capacity of microglial cells to migrate and to phagocyte fibrillar amyloid beta peptides increased upon NE stimulation in a concentration dependent manner (10 nM-10µM). Similary, DSP4 induced LC degeneration and NE depletion of APP transgenic mice resulted in a pronounced increase of mRNA and protein levels of cytokines, glial fibrillary acidic protein, inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2). Immunohistochemical evaluation verified a close relationship of Aβ deposits and the expression of inflammatory molecules in micro and astroglia. Confocal immunohistochemistry revelead that iNOS was expressed by microglial cells in APP control mice, while NE depleted APP mice showed a robus neuronal expression of iNOS. COX2 was predominantly expressed by IB4 positive microglial cells. NE depleted APP transgenic mice showed more Aβ1-40 and 1-42 deposits within the hippocampus and frontal cortex compared to APP transgenic mice with an intact NE innervation. Confocal analysis of microglial cells and Aβ revealed that the number of colabelled cells was significantly higher control APPV717I transgenics compared to NE depleted mice, suggesting that NE levels may modulate microglial phagocytosis also in vivo. Taken together this may indicate that LC degeneration and the subsequent decrease of NE concentrations facillitate the inflammatory reaction of microglial cells. At the same time, impaired microglial clearance may significantly contribute to Aβ deposition in AD.

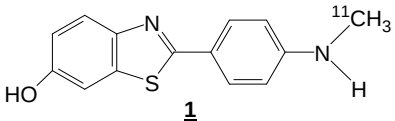
DEVELOPMENT AND PRECLINICAL EVALUATION OF A FLUORINE-18 LABELLED TRACER FOR ALZHEIMER'S DISEASE

K. Serdons[1], K. Van Laere[2*], P. Dupont[2], R. Vandenberghe[3], M. Vandenbulcke[4], H. Kung[5], G. Bormans[1], A. Verbruggen[1*]

[1]Laboratory of Radiopharmacy, University of Leuven, Leuven, Belgium; [2]Division of Nuclear Medicine and [3]Department of Neurology and [4]Department of Psychiatry, U.Z. Gasthuisberg, Leuven, Belgium; [5]Department of Radiology, University of Pennsylvania, Philadelphia, United States

** presenting authors*

The availability of a radiopharmaceutical with high affinity for amyloid plaque deposits and efficient blood-brain barrier passage would allow non-invasive *in vivo* imaging and diagnosis of Alzheimer's disease (AD) in an early stage and follow-up of treatment. Several 2-phenylbenzothiazole (PBTA) derivatives have been proposed for this purpose and one of them, 6-hydroxy-2-(4'-N-[^{11}C]methylaminophenyl)-1,3-benzothiazole (Pittsburgh Compound-B, [^{11}C]6-OH-BTA-1, PIB, **1**), is currently being clinically evaluated in different PET centers. In view of the practical advantages of fluorine-18 as compared to carbon-11, we have started a study for the development of a 2-phenylbenzothiazole radiolabelled with fluorine-18 on the 2-phenyl ring.



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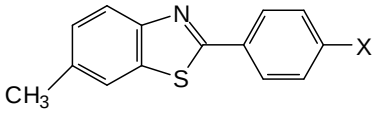


2 (X = F or ^{18}F)

A series of more than 20 PBTA's was synthesized with different substituents on the benzothiazole part and/or 2-phenyl ring in addition of a fluorine atom on the 2-phenyl ring. The affinity of the PBTAs for beta-amyloid present in post mortem brain homogenates of AD patients was measured using a competive assay with ^{125}I-IMPY as the tracer agent. Derivatives with an affinity better than 20 nM were synthesized in the fluorine-18 labelled form by an aromatic nucleophilic substitution reaction on the corresponding nitro precursors with ^{18}F-fluoride in the presence of Kryptofix.

The biodistribution of the radiolabelled PBTA's was studied in normal mice and rats with special focus on brain uptake shortly after injection and the rate of washout from normal brain (ratio brain activity 2 min/60 min). For selected compounds the profile of metabolism in plasma and brain was studied.

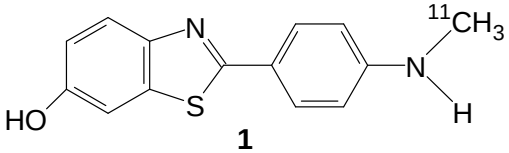
On the basis of the results, 6-methyl-2-(4'-[^{18}F]fluorophenyl)-1,3-benzothiazole (**2**) was selected as the most promising agent for preclinical evalution in a human study. An extended (14 days) single-dose toxicity study according to the microdosing concept was performed in rats and in addition, genotoxicity was evaluated using an Ames test and a micronucleus test. Approval from the local Ethical committee and the national Health authorities for a preclinal study was obtained on the basis of a full investigational medicinal drug dossier. The aims of this preclinical study were the establishment of the biodistribution profile and radiation dose of **2** in humans, an evaluation of its usefulness for diagnosing amyloid deposits in AD patients and comparison of **2** with PIB for diagnosis of AD. The first results of the human biodistribution, dosimetry and brain PET studies will also be presented.

2-(3'-[^{11}C]METHYLAMINO-4'-AMINOPHENYL)-1,3-BENZOTHIAZOLE AS POTENTIAL AMYLOID IMAGING AGENT

K. Serdons[a], C. Landeloos[a], C. Terwinghe[b], P. Vermaelen[b], K. Van Laere[b], H. Kung[c], G. Bormans[a], A. Verbruggen[a]

[a]Laboratory of Radiopharmacy, University of Leuven, Leuven, Belgium; [b]Department of Nuclear Medicine, U.Z. Gasthuisberg, Leuven, Belgium; [c]Department of Radiology, University of Pennsylvania, Philadelphia, United States

Introduction. Alzheimer's disease (AD) is a neurodegenerative disorder which causes progressive loss of memory and other cognitive functions and is characterized by the deposition of amyloid plaques in the brain. The availability of a radiopharmaceutical with high affinity for such plaque deposits and good blood-brain barrier passage would allow non-invasive in vivo imaging, early diagnosis and follow-up of treatment in AD. Several radiolabelled 2-phenylbenzothiazole derivatives have been proposed for this purpose and one of them, 6-hydroxy-2-(4'-N-[11C]methylaminophenyl)-1,3-benzothiazole (Pittsburgh Compound-B, [11C]6-OH-BTA-1, PIB, 1), is currently being clinically evaluated.1 As part of an ongoing project towards the development of radiolabelled 2-phenylbenzothiazoles with improved in vivo characteristics, we have now developed and evaluated a diamino carbon-11 labelled PIB derivative (2) as a new potential in vivo positron emission tomography (PET) tracer for Alzheimer's disease.



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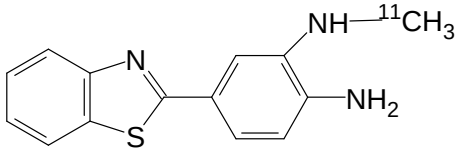

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Materials and methods. The affinity (Ki) of the 'cold' compound 2-(3'-methylamino-4'-aminophenyl)-1,3-benzothiazole (3) for post mortem brain homogenates of AD patients containing amyloid was determined following a described procedure2. Radiolabelling to prepare 11C-labelled 2 was realized by using methyl triflate (3 min, 70 °C). The log P value of RP-HPLC purified 2 was determined in octanol/0.025M phosphate buffer pH 7.4. Biodistribution of 1 and 2 was studied ex vivo at 2 and 60 min p.i. in normal mice and in vivo in a normal rat with a µPET camera.

Results. Affinity of 3 for post mortem human AD brain homogenates was 6.0 nM. The log P value for 2 was 1.5. In normal mice, brain uptake was high at 2 min p.i. (3.20 % ID, 10.50 % ID/g) and wash-out from normal brain was rapid (at 60 min p.i.: 0.08 % ID, 0.27 % ID/g). The µPET study confirmed the high brain uptake and fast wash-out of this compound.

Conclusion. The new 2-(3'-methylamino-4'-aminophenyl)-1,3-benzothiazole shows good affinity for human brain homogenates containing amyloid. The Ki-value is in the same order as that of 1 which is 2.8 nM. The biodistribution and µPET study show favorable characteristics. The new carbon-11 labelled phenylbenzothiazole has a higher brain uptake than PIB (1 at 2 min p.i.: 1.08% ID, 3.60% ID/g). Brain wash-out (% ID cerebrum 2 min/% ID cerebrum 60 min) of 2 in normal mice is 6 times faster than that of PIB. The excellent characteristics of the new 11C-labelled compound suggest that it is a promising candidate for in vivo visualization of amyloid.

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IMAGING THE CAUSES AND CONSEQUENCES OF INHALANT ABUSE

Schiffer WK[1], Carrion J[1], Lee DL[1], Mirrione MM[1], Benveniste H[2], Fowler JS[1], Ferrieri R[1] and Dewey SL[1]

[1]Chemistry and [2]Medical Departments, BNL, Upton, NY

Inhalant abuse in first and third world countries has now reached epidemic proportions. This is significant given that the primary abusing population consists of children between the ages of 12 and 16, and that members of this vulnerable population are 46-times more likely to become poly-substance abusers. Solvents containing aliphatic, aromatic and halogenated hydrocarbons readily penetrate the CNS and produce a euphoria with rapid onset and short duration. Certain products, such as those containing toluene and benzene, are preferred. It is not known what neurochemical properties underlie this preference, nor have the short or long term effects on brain function been well established.

We have developed an animal model of inhalant abuse using the conditioned place preference (CPP) paradigm in a modified inhalation chamber. Over time, animals who inhale toluene vapors in a distinct chamber show a marked preference for the toluene-paired chamber when given a choice. In agreement, small animal PET studies demonstrated a significant reduction in [^{11}C]-raclopride binding produced by a bolus dose of toluene. FDG microPET studies on animals prior to and following a conditioning regimen of inhaled toluene show a regionally specific decrease in cortical glucose metabolism that persists throughout the conditioning period, but rebounds to normal levels two months after cessation. On the other hand, animals paired to inhaled acetone fail to develop a preference for the acetone paired chamber, and acetone has little impact on cortical glucose metabolism. This and related evidence supporting the specific abuse liability of different solvents will be presented.

Neuroimaging studies of radiolabeled inhalants in rodents and primates suggest that the reinforcing properties as well as the damage are linked to specific pharmacokinetic profiles and regional distributions. Primate and rodent PET data showing the distribution and kinetics of [^{11}C]-toluene, [^{11}C]-butane and [^{11}C]-acetone will be presented. This data provides evidence of a correlation between the pharmacokinetic properties of these inhalants and their ability to produce a place preference. Significant accumulation of toluene in white matter led us to test the hypothesis that exposures of toluene sufficient to produce a place preference also alter brain structure. Serial scans using magnetization transfer contrast MRI (MT-MRI) in animals prior to and following a reinforcing exposure to toluene demonstrated significant changes in brain white matter. Histological data supported the decline in MTR signal. When imaged with FDG, the same animals had significantly decreased cortical metabolism. Voxel-based image analysis using Statistical Parametric Mapping (SPM) revealed several brain regions in which the reduction in metabolism significantly correlated with the degree of tolerance to toluene (measured by locomotion during toluene pairings).

In conclusion, accumulation of carbon-11 labeled inhalants in lipid-rich regions of the brain is directly associated with pathology on MR data from the same animals. There is a strong relationship between the pharmacokinetic and pharmacodynamic properties of inhalants and their reinforcing properties as well as their physiologically toxic effects.

NEW APPROACH FOR MEASURING P-GLYCOPROTEIN IN THE BLOOD-BRAIN BARRIER, SYNTHESIS AND BIODISTRIBUTION OF [^{11}C]LANIQUIDAR.

G. Luurtsema, R.C. Schuit, R.P. Klok, J.D.M. Herscheid, J. Verbeek, J.E. Leysen, A.A. Lammertsma, A.D. Windhorst.

Department of Nuclear Medicine and PET Research, VU University Medical Center, Amsterdam, The Netherlands.

Introduction

P-glycoprotein (P-gp) at the blood-brain barrier (BBB) may play a role in the aetiology of several neurological diseases and in drug transport across the BBB (1.2).

A promising method was developed with (R)-[11C]verapamil and Positron Emission Tomography (PET) to study the role of functional P-gp in the BBB (3) and recently, the first application has been described (4) . For some applications however, there are inherent limitations associated with a tracer, which is a substrate for P-gp. In particular, it will be difficult to measure overexpression of P-gp, which might be, at least in part, responsible for drug resistance in e.g. epilepsy (2). In such a case, uptake will be even lower compared to normal tissue where it is already low, resulting in poor counting statistics.

An alternative strategy might be the labelling of the third generation P-gp inhibitor, laniquidar (R101933). Apart from its high potency and specificity for P-gp, this antagonist is not a substrate for P-gp, but binds with high affinity to the pump itself.

Aim

The purpose of the present study was to assess whether it is possible to synthesize the third generation inhibitor laniquidar with carbon–11 as a ligand for PET studies and study its biodistribution in normal rats.

Results

[11C] Laniquidar was obtained by methylation of its free acid precursor R102207. Addition of [11C]CH3I to 2.0 mg of this precursor dissolved in 300 µL of DMF containing 1.2 µL of 60% TBAH gave after 2 minutes reaction time at 60oC a radiochemical yield of 30% (decay corrected). The product was purified from the reaction mixture by HPLC (µBondapak 7.8x300; acetonitril/water/TFA 68/32/0.1, 4.5 ml/min). The collected fraction containing [11C]laniquidar was diluted with 40 ml water and this mixture was subsequently passed over a tC18 Seppak. After washing the Seppak with 20 ml of water for injection, [11C]laniquidar was eluted from the Seppak with 1.5 ml of sterile ethanol and subsequently 14 ml of a sterile solution of 2.5% polysorbate 80 in saline. The final solution was transferred over a 0.22 µm filter (Millex GV), yielding a sterile, pyrogen free solution of [11C]laniquidar with a (radio)chemical purity of > 98%

Male Wistar rats were injected with 20 MBq of [11C]laniquidar (S.A. > 18.5 GBq/ µmol) via a tail vein, and 5, 15, 30 and 60 minutes after injection the rats were sacrificed and several tissues and distinct brain regions were dissected and counted for radioactivity.

Conclusion

[11C]laniquidar can be reproducibly synthesised in sufficient amounts for in vivo PET studies. Uptake of the tracer was found in brain, kidney, liver, spleen and lung. Further studies are needed to assess whether the uptake of the tracer is specific for P-gp. This new approach for studying P-gp in vivo will be further evaluated.

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MOLECULAR AND STRUCTURAL NEUROIMAGING IN CEREBELLAR ATAXIA

E. Salvatore[1], A.Varrone[2], G. De Michele[1], A. Filla[1], M. Salvatore[2,3], S. Pappatà[2] .

[1]Dep. of Neurological Sciences, Univ. Federico II, Napoli, Italy; [2]Biostructure and Bioimaging Inst, CNR, Napoli, Italy; [3]Dep. of Biomorphological and Functional Sciences, Univ. Federico II, Napoli, Italy.

Introduction: Cerebellar ataxias represent a wide heterogeneous group of neurodegenerative diseases, most of which are hereditary. Autosomal recessive forms have usually an early onset, while autosomal dominant forms have usually an adult onset. Non-familial forms refer usually to idiopathic late-onset cerebellar ataxia (ILOCA) or multiple system atrophy, cerebellar type (MSA-C). Molecular biology discoveries let us to identify 27 different forms of autosomal dominant ataxias, whose prevalence is about 3/100.000 (www.geneclinics.org). Cerebellar signs are the main features. However, multiple neurological signs are present so variably according to genotype that is not easy for clinicians to identify the appropriate genetic test only by neurological exams. Clinical variability corresponds to neuropathological variability with heterogeneous involvement of olivo-ponto-cerebellar system and spinal cord mainly, as well as cerebral cortices, basal gangli and thalamic nuclei.1 Overall, ataxic disorders seem to share some clinical and neuropathological findings while presenting specific hallmarks. Which is the contribution of common pathogenetic mechanisms is still an unresolved issue, but an accurate diagnosis is important for genetic counselling and prognosis. Combining molecular and/or structural neuroimaging with genetics may provide a unique tool to better characterize patterns of brain abnormalities associated to both phenotype and genotype. This could also improve the classification, the understanding of the pathophysiology and/or pathogenetic mechanisms, the prediction of progression and/or evolution of disease.

Objective: our work aims to combine molecular genetic and neuroimaging to define neurochemical and structural changes in homogeneous groups of patients with sporadic and familial cerebellar ataxia. More specifically we have used dopamine transporter (DAT) SPECT imaging to assess dopaminergic nigrostriatal function/integrity in familial spinocerebellar ataxia type 2 (SCA2) and type 17 (SCA17) and sporadic late onset cerebellar ataxia (ILOCA) as well as MSA with predominant cerebellar involvement. We also used diffusion-weighted imaging (DWI) to identify microstructural alterations in both subtentorial and supratentorial regions in SCA2 patients. Finally we also tested possible relationships between neurochemical/microstructural changes and clinical findings in these patients.

Methodology: [123I]FP-CIT SPECT (E.CAM-dual-headed/128x128 matrix/voxel size: 3.9x3.9x3.9mm/Butterworth filter-cut-off 0.5-order 10/Chang algorithm-□=0.06 cm-1; images spatially normalised in the MNI space (SPM'99; voxel size:4x4x4mm), and analysed with ROIs) was performed in 6 SCA2 (5M/1F, 48±5yrs, disease duration:10±4yrs), five SCA17 (4M/1F, 42±11yrs, disease duration:5±5yrs), 9 ILOCA (7M/2F, 52±9yrs, disease duration:8±5yrs), 9 MSA-C patients (2M/7F, 59±11yrs, disease duration:3±2yrs) and 9 controls (7M/2F, 44±16yrs). DWI-MRI (Philips 1.5T device; Single shot EPI ; 3 directions (y,x,z); 3 b values (0,500,1000); 40 slices-3mm, ADC maps) studies were recorded in 13 SCA2 patients (9M/4F, age:50±12yrs, disease duration:11±5yrs), and 15 controls (10M/5F, age: 49±14). Apparent diffusion coefficient (ADC) values were collected from the regions of interest (ROI), manually designed on B0 images, in frontal white matter (FWM), posterior white matter (PWM), caudate, putamen, thalamus, pons, middle cerebellar peduncles (MCPs) and cerebellar white matter (from superior and inferior slices, cer-WM). Statistical analysis in [123I]FP-CIT SPECT studies was used ANCOVA(age as covariate)/Tukey's post-hoc test. Significance was set at the p=0.05. In DWI studies t-test for differences between groups and Spearman's rho for ADC and clinical correlations were performed. All patients were clinically evaluated with ataxias scales.

Results. Age was comparable between groups. [123I]FP-CIT SPECT studies showed a significant nigrostriatal deficit in SCA2, in patients with no extrapyramidal signs. In SCA17 patients the nigrostriatal deficit was present in patients with fully developed phenotype, but it was normal in subjects in presymptomatic and early phase of the disease (a patient showing only focal dystonia). In both SCA2 and SCA17 striatal DAT density also seemed to be correlated with the severity of cerebellar ataxia.2,3 The striatal DAT density in ILOCA patients was not different from that of controls (2.58±0.64 vs. 2.89±0.38). However, in 2/9 (22%) ILOCA patients DAT density was decreased by 39% in the caudate and by 45% in the putamen. These findings are quite different from those of MSA-C patients in which the DAT density was significantly decrased (1.73±0.44) in all striatal regions as compared with controls ($p<0.01$) and in ipsilateral putamen and contralateral caudate and putamen ($p<0.05$) compared with ILOCA. Average striatal DAT density was reduced by ≥20% in all MSA-C patients. In 5/9 (55%) of them, caudate DAT density was decreased by 45%, while in 6/9 (67%) of them putamen DAT density was decreased by 50%. DWI in SCA2 showed a significant increase ADC

(x10-3 mm2/sec) in cer-WM (superior cereb: 0.922±0.060 vs 0.683+-0.039, p<0.0001; inf cereb: 0.8751±0.059 vs. 0.703±0.047, p<0.0001), MCPs (1.004±0.073 vs. 0.729±0.034, p<0.0001) and pons (0.896±0.0659 vs. 0.712±0.059, p<0.001). In SCA2 significant positive correlations were found between disease duration and ADC values in MCPs (rho=0.758, p<0.01). Severity of Ataxia correlated with FWM (rho=0.740, p<0.01) and cereb-WM (rho=0.611, p< 0.05).

Discussion. In vivo neurochemical and microstructural imaging techniques may help in understanding the pathophisiology, the evolution and differential diagnosis of cerebellar ataxias. Our results showed that: 1) the nigrostriatal dopaminergic system is involved in SCA2 albeit the absence of pakinsonism2 while it is only mildly altered in SCA17 where it does not occur in the earliest disease stages. In both SCA2 and SCA17 the dopaminergic dysfunction/deficit seems to be correlated to the severity of cerebellar clinical signs, suggesting a possible contribution of nigrostriatal degeneration to the severity and/or progression of diseases. 2) in MSA-C there is a marked impairment of the nigrostriatal dopaminergic system albeit the prevalence of cerebellar symptoms; 3) the nigrostriatal deficit can be detected in a small percentage of ILOCA patients suggesting that this could be an early sign of development of MSA; 4) Microstructural changes in SCA2 mainly involves subtentorial regions. The severity of microstructural changes in both subtentorial and supratentorial (FWM) regions is correlated with the severity of cerebellar clinical deficit and/or disease duration.

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AMYLOID-PLAQUE IMAGING IN NON-ALZHEIMER DEMENTIA

Alexander Drzezga MD1*; Timo Grimmer MD2*; Gjermund Henriksen PhD1, Isabelle Stangier MS1, Robert Perneczky MD2, Janine Diehl-Schmid MD2, Chester A. Mathis PhD3, William E. Klunk MD PhD4, Hans-Jürgen Wester PhD1, Markus Schwaiger MD1 and Alexander Kurz MD2

1Department of Nuclear Medicine; Technische Universität, München; 2Department of Psychiatry and Psychotherapy; Technische Universität, München; 3 Department of Radiology; University of Pittsburgh, U.S.A; 4 Department of Psychiatry, Western Psychiatric Institute & Clinic, Pittsburgh, U.S.A

Background: Semantic dementia (SD) is a rare clinical syndrome, assigned to the group of frontotemporal lobar degenerations (FTLD). Histopathological analysis has not revealed the deposition of amyloid-plaques in the majority of SD-cases, in contrast to dementia of the Alzheimer type (AD). However, some overlap of clinical symptoms and imaging findings between AD and SD has been shown and a reliable differentiation of the underlying pathology can not be guaranteed based on clinical examination alone. Our aim was to determine, whether differences between AD and SD can be found by means of in vivo amyloid-plaque imaging, providing complementary information to results obtained by measurement of cerebral glucose metabolism.

Methods: AD and SD patients, matched for gender, age and overall degree of cognitive impairment, were recruited using established clinical criteria. Cerebral glucose metabolism was examined with [F-18]Fluorodeoxyglucose (FDG)-PET, cerebral amyloid-plaque density was assessed using [C-11]6-OH-BTA-1 (PIB)-PET. A volume-of-interest analysis (VOI), using the cerebellum as a reference region, and voxel-based statistical group comparisons (SPM2) were carried out between the patient groups (FDG- and PIB-PET data) and a group of healthy controls (FDG-PET data).

Results: Characteristic patterns of hypometabolism could be demonstrated in both clinically defined AD and SD (AD: bilateral temporoparietal and frontal cortex; SD: left>right temporal, frontal mesial cortex) with some regional overlap, particularly in temporal cortices. In contrast, strong [C-11]PIB amyloid-plaque tracer binding was observed only in patients with AD in bilateral temporoparietal, frontal and posterior cingulate cortex and the precuneus. This increased amyloid-plaque deposition, observed in AD, clearly extended the metabolic differences between the groups, even in regions with comparable metabolic deficits.

Conclusion: These findings support the common notion hat neuronal dysfunction is associated with amyloid-plaque deposition in AD. In contrast, in the examined cases of typical SD, neuronal dysfunction was apparently based on pathology other than amyloid-plaque deposition. Amyloid-plaque imaging may be valuable to clearly characterize dementias in vivo, based on the underlying pathology rather than on neuropsychological findings. This may be important for definition of individual prognosis and for the selection of patients for scientific trials.

MRI MEASURES OF INFLAMMATION AND DEGENERATION IN MULTIPLE SCLEROSIS

Maria A Rocca, Massimo Filippi

Neuroimaging Research Unit, Department of Neurology, Scientific Institute and University Hospital San Raffaele, Milan, Italy.

Conventional magnetic resonance imaging (cMRI) is widely used for diagnosing multiple sclerosis (MS) and monitoring disease activity and evolution in natural history studies and clinical trials. However, the correlation between cMRI and clinical findings is far from being strict and such a discrepancy is even more evident when moving from the setting of large-scale studies to the management of individual patients. Among the reasons for this "clinical-MRI paradox", a major role has been attributed to the limited specificity of T2-weighted imaging to the heterogeneous pathological substrates of MS and to its inability to quantify the extent of damage in the normal-appearing tissues. In addition, although Gd-enhanced MRI is able to image the inflammatory component of this disease, it also lacks pathological specificity to the various tissue changes, such as demyelination and axonal loss, which occur at the site of inflammation and which may influence the clinical outcome of MS.

Modern quantitative MR techniques have the potential to overcome some of the limitations of cMRI. Metrics derived from magnetization transfer and diffusion-weighted MRI enable us to quantify the extent of structural changes occurring within and outside macroscopic MS lesions with increased pathological specificity over cMRI. MR spectroscopy can add information on the biochemical nature of such changes, with the potential to improve significantly our ability to monitor inflammatory demyelination and neuroaxonal injury. Functional MRI might provide new insight into the role of cortical adaptive changes in limiting the clinical consequences of structural damage. The development and application of cell-specific imaging, as well as the acquisition of Gd-enhanced MRI in association with other MR techniques, thought to be more pathologically specific, are also likely to go some way towards overcoming some of the aforementioned limitations.

The application of modern MR techniques is changing dramatically our understanding of how MS causes irreversible disability, and is showing that MS is more than an inflammatory-demyelinating condition of the white matter of the central nervous system.

Q-SPACE ANALYZED DIFFUSION WEIGHTED MRI IN MULTIPLE SCLEROSIS

Henrik Lund and Lars G. Hanson

Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Kettegaard Allé 30, 2650 Hvidovre, Denmark.

Introduction: Multiple sclerosis (MS) is a chronic, inflammatory, autoimmune, demyelinating disease of the CNS. The pathological processes cause a structural breakdown of the tissue within the CNS and thus alter the microscopic movement of water (diffusion).

While the diffusion of water in pure water is random the diffusion of water in tissue is partly restricted due to the structures in the tissue. As diffusion can be measured /in vivo/ by diffusion weighted magnetic resonance, it is possible to obtain structural information on the various biological barriers and compartments. The axons with its surrounding myelin membranes are particularly interesting in this regard as 1) the myelin membranes are difficult for water to penetrate, 2) axons and their myelin sheaths are highly important for the functionality of the CNS and 3) specifically the myelin sheaths are targeted by the pathological processes of MS.

Normal diffusion tensor imaging (DTI) analysis gives one (apparent) diffusion coefficient (ADC) based on the assumption that the sample behaves as a perfect gaussian distribution. However, while an increased ADC does not tell us whether the viscosity is lowered or whether the sample has less barriers the q-space analysis provides a size distribution of the tissue structures and can potentially give clinically relevant information on the breakdown of the structures.

In the present study we aim at showing that q-space imaging can provide clinically relevant information in MS at scanning times suitable for the clinic.

We know of only one other group who has publiced q-space analyzed diffusion data of multiple sclerosis (Assaf et al., 2002). They conclude that compared to DTI, q-space analyzed data to a greater extend explain differences seen between lesions and normal appearing brain tissue in MS-patients and normal tissue in healthy subjects.

The number of patients in our work is larger, we use higher field strength, and it is widely known that diffusion properties of normal appearing brain tissue are changed in MS. Hence, we find it reasonable to assume that we will find abnormal tissue properties based on our diffusion measurements and the q-space analysis.

Patients: Two groups of patients are scanned: 1) a group of 40 patients scanned three times with intervals of three months and 2) a group of 60 patients scanned once.

Method: The measurements for the q-space imaging are in principle similar to that of traditional diffusion weighted images. However, q-space analysis goes beyond the implicit assumption made in normal DWI/DTI analysis of tissues having a single directionally dependend diffusion coefficient. Instead the analysis is cast into a general framework where higher order moments of the probability distribution are included and expressed, e.g., by the 4-dimensional kurtosis tensor. The shape of this probability distribution reflects the structures of the sample. The number of independent, higher order moments is limited to a few measurable quantities by imposing structural contraints.

Results: A protocol providing diffusion weighted images suited for q-space analysis for a range of diffusion times and q-values in a clinically feasible measurement time has been developed. Results of the analysis are only preliminary.

Discussion: The number of independent, higher order moments characterising diffusion increase dramatically when the diffusion tensors of high rank are included. So do the noise sensitivity and the measurement time unless measures are taken to extract few, well-characterized and interpretable parameters. It is too early to say whether these parameters provide clinically relevant information.

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METABOLIC-DOPAMINERGIC MAPPING OF THE QUINO-LINIC ACID RAT MODEL FOR HUNTINGTON'S DISEASE

C. Casteels[1], E. Martinez[2], L. Camon[2], K. Goffin[1], N. de Vera[2], G. Bormans[3], V. Baekelandt[4], A. Planas[2] and K. Van Laere[1]

[1] Division of Nuclear Medicine, K.U.Leuven, Belgium; [2] IIBB-CSIC, IDIBAPS, Barcelona, Spain; [3] Laboratory for Radiopharmacy and [4] Neurobiology and Gene therapy, K.U.Leuven, Belgium

Phone +32(0)16.34.37.15; Fax +32(0)16.34.37.59; E-mail cindy.casteels@uz.kuleuven.be

We have characterized in vivo metabolic/dopamine D2 receptor changes in the quinolinic acid (QA) lesion rat model for Huntington's disease (HD) and investigated metabolic alterations in correlation to disease severity. **Methods:** 20 Wistar rats (10 QA, 10 PBS sham) were investigated. QA and PBS were stereotactically injected in the left caudate-putamen (CPu). MicroPET acquisitions were conducted on a FOCUS 220 system at 15-26 weeks post lesioning using 18 MBq of ^{18}F-FDG and ^{11}C-Raclopride. Data were spatially normalized to Paxinos space and analyzed using SPM2. **Results:** Glucose metabolism and D2 receptor binding were reduced in the ipsilateral CPu by 35% and 77% respectively ($p_{height} \leq 2.10^{-11}$), while an increase for these markers was seen in the contralateral CPu (>6%, $p_{height} \leq 2.10^{-4}$). Relative metabolism was also increased in the contralateral hippocampus, thalamus and sensory-motor cortex ($p_{height} = 1.10^{-6}$). Correlation analysis revealed a positive relation between D2 impairment and metabolic activity in the ipsilateral CPu ($p_{height} < 1.10^{-4}$, small volume corrected). **Conclusion:** In vivo cerebral microPET mapping in QA rats points to a functional plasticity with recruitment of parallel motor circuits to compensate for the functional deficit of the ipsilateral striatocortical motor loop.

VIEWING LIGHT BY MRI BASED ON PHOTOCHEMICAL AND HEMODYNMIC TISSUE RESPONSES TO VASCULAR-TARGETED PHOTODYNAMIC THERAPY (VTP)

Catherine Brami[1], Noa Madar-Balakirski[1], Iddo Pinkas[2], Avigdor Scherz[2] and Yoram Salomon[1]

The Departments of Biological Regulation[1] and Plant Sciences[2] The Weizmann Institute of Science Rehovot, 76100, Israel

Introduction: Vascular-targeted photodynamic therapy (VTP) is an anti-vascular tumor treatment whereby the blood supply to the tumor is disrupted within minutes initiating hypoxia, with subsequent necrosis and tumor eradication (1, 2). This treatment modality is presently in clinical trials in phase II/III for treatment of prostate cancer therapy and in phase II for therapy of age related macular degeneration using the Pd-bacteriochlorophyll derivatives WST09 and WST11 as respective photosensitizer (PS) drugs. VTP is based on the in situ illumination of the i.v. injected PS. When circulating through the targeted illuminated blood vessel network, the PS is instantly photosensitized, triggering a confined burst of reactive oxygen species (ROS) upon local consumption of blood oxygen. The resulting rise in blood deoxy-hemoglobin (Hb, an endogenous paramagnetic Magnetic Resonance Imaging (MRI) contrast marker) was shown by us to generate changes in image contrast based on Blood Oxygen Level Dependent (BOLD) MRI (3). The ability to map the illuminated target area during VTP was examined in this study.

Objective: To develop an in vivo BOLD MRI technique for real time imaging of illuminated tissue zones.

Methodology and Results: Using WST11, we show in this study that limited by the density of the vascular network, photosensitized (ps) MRI allows noninvasive in-vivo detection with spatio-temporal information of an illuminated image displayed on rat subcutaneous tumors or normal striated muscle. Light image registration was computed by correlating BOLD contrast responses to a triggering illumination paradigm. The illuminated areas were deduced from regions with contrast enhancement of 55±46 fold (n=15) relative to the surroundings, using light energies from 1.7 to 75 J/cm2. The underlying photochemical and hemodynamic mechanisms and the MRI based technique of light detection will be discussed.

Discussion: The discovered phenomenon opens exciting new experimental possibilities such as using vascular networks as screens for dynamic mapping of infiltrating light; probing optical tissue transparency; or correlating hemodynamic response maps to light-induced hypoxic sinks from the ensuing BOLD MRI changes. Moreover, psMRI is emerging as a possible tool for non-invasive interactive light guidance, follow up and endpoint determination of photodynamic interventions.

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MULTI-MODAL IMAGING OF FUNCTIONAL BRAIN ACTIVATION – METHODOLOGICAL EVALUTATION AND PRELIMINARY RESULTS

[1,2]H. Backes, [1,2]Y. Waerzeggers, [2]U. Himmelreich, [2]R. Graf, [2]G. Mies, [2]M. Hoehn, and [1,2,3,4]A. Jacobs

[1]Department of Neurology at the University of Cologne, Cologne, Germany; [2]Max-Planck-Institute for Neurological Research with Klaus-Joachim-Zülch-Laboratories of the Max-Planck Society, Cologne, Germany; [3]Department of Neurology at the Klinikum Fulda, Fulda Germany; [4]Center for Molecular Medicine at the University of Cologne, Cologne, Germany

Phone +49 221 478 87960; Fax +49 221 478 6052 ; E-mail backes@nf.mpg.de

Several diseases have a strong impact on brain functionality. In order to examine disease severity or temporal course it is useful to develop non-invasive methods that measure brain functionality in terms of the response of the brain to a defined external stimulus.

In this study performed on rats we compare the effects of electrical fore-paw stimulation on the glucose consumption measured by 18F-FDG positron emission tomography (PET) and 18F-FDG autoradiography (AR) with the change of cerebral blood flow measured by MRI using BOLD effect. Although AR is the gold standard for the determination of glucose consumption, for the establishment of a long term functional study AR is not a useful candidate. We show that PET is capable of locating the stimulated part in the frontal cortex in the same way as AR and can therefore replace AR in its ability of measuring glucose consumption.

Further more the activated brain region displayed in the 18F-FDG PET matches the region with increased BOLD effect on visual MRI. The complementary information obtained by both imaging modalities will increase the understanding of disease pathophysiology.

IMAGING OF NEURODEGENERATION AND STEM CELL MIGRATION IN RODENT MODELS USING LENTIVIRAL VECTORS

Abdelilah Ibrahimi and Veerle Baekelandt

Division of Molecular Medicine and MoSAIC, K.U.Leuven, Leuven, Belgium.

Lentiviral vectors are powerful tools for gene delivery into the central nervous system since they stably integrate non-dividing cells such as neurons. In addition to the promising therapeutic applications, lentiviral vectors (LVs) have been used to generate new disease models by means of locoregional overexpression or inhibition of disease-correlated genes in the adult brain, which brings more insights in understanding the neurodegeneration process.

We are conducting stereotactic injections of HIV-1-derived lentiviral vectors to overexpress Parkinson's disease-related genes (e.g. α-synuclein) in the brain of rats and mice and to mark endogenous neuronal stem cells. Besides overexpression, we also knock-down genes through the expression of short-hairpin RNA based lentiviral vectors. The outcome of in vivo overexpression or inhibition of Parkinson's disease-linked genes is analysed by non-invasive imaging, histopathology and behaviour.

At present, our group is developing and validating lentiviral vectors encoding marker genes for optical bioluminescence imaging and MRI with the rationale of non-invasive imaging of the transgene and stem cell migration in the rodent brain. Some of the progress with these imaging vectors encoding firefly luciferase or ferritin will be discussed.

GENE TRANSFER AND IMAGING IN THE CNS

Nicole Déglon

Atomic Energy Commission (CEA), Institute of BioMedical Imaging and MIRCen program, Orsay, France

Tools allowing non-invasive assessment of disease-specific biological and molecular processes with high spatial and temporal resolution are critically needed to identify underlying degenerative mechanisms and evaluate therapeutic candidates for CNS disorders. The ability of viral vectors to efficiently transduce postnatal neurons and glia provide a unique opportunity to express imaging genes, such as fluorescent proteins into relevant cellular targets and perform high resolution live cell imaging and investigate CNS structure and functions. In addition, viral vectors are extensively used in experimental models to overexpress or inactivate genes of interest and reveal disturbances in neuronal and glial functions. Finally, considerable efforts have been made, over the last years, to develop novel treatments for neurodegenerative diseases based on gene therapy approaches. Examples, illustrating the challenges and impact of viral gene transfer on in vivo imaging of diseased nervous system will be presented.

VISUALISATION OF DENDRITIC CELLS AND THEIR PROGENITORS BY MRI

U. Himmelreich

In vivo NMR laboratory, Max-Planck-Institute for Neurological Research Cologne, Germany

Background:

Gd-chelates and ultra-small paramagnetic iron oxide particles (USPIOs) are suitable for the visualization of deposits of cells in vivo. We have studied their suitability for the visualization of dendritic cells and the visualization of intracellular enzyme activity.

Methods:

Flt3+CD11b+progenitor cells and dendritic cells were isolated from mice. Labeling of cells was performed using USPIOs (endorem as well as sinerem) as well as Gd-DTPA. In addition, labeling was performed using a responsive contrast agent consiting of a Gd-DTPA chelate linked with two long fatty acid chains (C17H35) through ester bonds. This insoluble Gd-DTPA-FA complex can be activated by lipase activity and had a relaxivity of zero in the inactive state and 4.7 mM-1 s-1 after activation, respectively.

MRI: T1- and T2*-weighted MR images were acquired using a Bruker Biospin 7.0 Tesla small animal scanner equipped with an actively shielded gradient sets of 200 mT m-1 using 3D gradient echo sequences (FLASH) with TR=60ms (T1w) and 150ms (T2*w), TE=5ms(T1w) and 20ms (T2*w), 70º (T1w) 30º (T2*w) pulse, FOV= 3x3x1cm (animal model) and 4.5x4.5x1cm (agar phantoms), the isotropic spatial resolution was 78 □m for phantoms and 50 □m for animal experiments. For rf irradiation and signal detection custom-built coils were used. A 5-cm-diameter transmit-receive coil was used for agar phantoms and a 12-cm-diameter Helmholtz coil arrangement served for rf excitation with a 3.0 cm diameter surface coil for signal detection for animals. MR images were processed with the NIH software ‘Image J’.

Animal model: 10,000-1,000,000 cells suspended in 2 □l were implanted in normal Wistar rats (n=4) into the border between the cortex and the corpus callosum (0.5 mm anterior, 3.0 mm lateral to bregma, 2.0 mm ventral from the dural surface) using sterotactic injection. Animals were imaged immediately after implantation and 4-14 days thereafter.

Results and Discussion:

Cell detectability was highest using USPIOs. The visualisation of cells with Gd-chelates depended on the route of uptake. Incubation of cells with Gd-chelates resulted in saturation of the R1 relaxation rate for high concentrations of Gd_DTPA (>20mM). Electroporation of cell suspensions containing up to 100mM Gd resulted in stable labeling of all cell-lines and no saturation of R1. Linking the Gd-DTPA complex to long aliphatic side chains results in a responsive contrast agent that can be activated by intracellular enzymes. We have tested this concept by using an ester link and activation of the chelate by intracellular lipase activity in dendritic cells. We were able to show that insoluble Gd-chelates are a suitable contrast agent for conditional activation by intracellular lipases. The chelate can easily be modified to be targeted by enzymes expressed during specific change of cell status. Such a system will then be suitable for functional cellular MR imaging. The concept was validated in animal models (ischemia in rats after MCAO).