

France-Australia Symposium on Nuclear Medicine

Methodology, Clinical Applications and
Pharmacology

Volume of Proceedings

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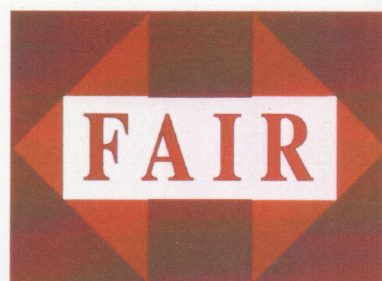
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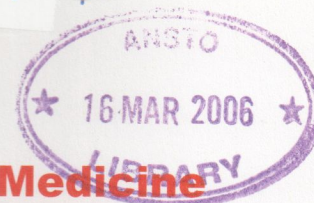


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France-Australia Symposium on Nuclear Medicine Programme

Opening of Symposium and Plenary Address

Chair, Professor Helen Garnett, Executive Director, ANSTO

0900 - 0930	Welcome and introduction by French Ambassador to Australia H.E. Pierre Viaux & Professor Judith Whitworth, Director of the John Curtin School of Medical Research, Australian National University.	Page 4 Page 6
0930 - 1030	Plenary Invited Lecture by Professor André Syrota, Director Health Sciences, French Atomic energy Commission (CEA). "Functional imaging with radionuclides: a vital tool for drug development and cell and gene therapies".	Page 8

Session #1 Imaging

1100 - 1130	"State of the art in both <i>in vitro</i> and <i>in vivo</i> aspects of Small animal Imaging" Prof. Bernard Mazière (Saclay), Prof. Denis Guilloteau (Tours)	Page 12
1130 - 1150	"Recent Developments in Instrumentation for Pre-Clinical Imaging Studies" Dr. Steve Meikle (Sydney)	Page 15
1150 - 1210	"Studies of nicotinic receptors in non-human primates using PET and SPECT." Dr. Michael Kassiou (Sydney)	Page 18
1210 - 1220	From academic research to the creation of Chelatec company. Dr. Anthony Loussouarn CHELATEC institute de biologie, Nantes.	Page 21
1220 - 1230	Profile of Bionexis Pharmaceuticals SA Dr. Françoise Russo-Marie, INSERM France.	Page 22

Session #2 France - Australia Collaborations

1230 - 1315	"Imaging cholinergic neurones with F-18 A85380 PET in the normal elderly and patients with Alzheimer's disease." (Christopher Rowe, Henri Touchon-Danguy, Gordon Chan, David Reutins) and CEA PET Centre, Service Hopital, Frederic Joliet, Orsay (Michel Bottlaender, Bernard Maziere, Sabina Pappata, Frederick Dolle).	Page 23
	"Development of Tracer for Multi Drug Resistance (MDR) exploration by Scintigraphy". Denis Guilloteau (INSERM U 316, CHU Tours) and Andrew Katsifis (ANSTO, Lucas Heights).	Page 26
	"Development of MPPF, a fluorine-18 labelled radiotracer for exploration of 5HT1A serotonergic system". Didier Lebars (CERMEP Lyon) and Henri Tochon-Danguy (Austin & Repatriation Medical Centre Melbourne).	Page 28
	"Improvement of AES (Affinity enhancement system) using a 188-Re-Hapten" a "FAIR" project. Laurence Morandeau (Nantes).	Page 30
	"Past, present and future collaborations - Orsay & Australia" Christian Loc'h. (Orsay)	Page 33

Session #3 Prognostic Developments in Cancer Treatment and Follow-up

1400 - 1420	"Value of Sestamibi for the prediction of resistance to primary chemotherapy". Prof. Jean Maublant (Clermont-Ferrand)	Page 36
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DEWEY

1420 - 1440	"From Structural to Biological Prognostic Stratification of Cancer: The New PET-Paradigm" Dr. Rod Hicks (Melbourne)* * Dr Rod Hicks was unable to present on the day. Instead, Dr J Harvey Turner (WA) presented "Radioimmunotherapy with Iodine-131 Anti-CD20 Chimeric Monoclonal Antibody (Rituximab) for Relapsed or Refractory Non-Hodgkins Lymphoma"	Page 38 Page 43
1440 - 1500	"Iodobenzamides, agents with an affinity for melanoma: applications to diagnosis, staging and therapy". Dr. Nicole Moins (Clermont-Ferrand)	Page 44
1500 - 1520	"Sentinel Lymph Node Biopsy: Clinical Relevance?" Dr. Robert Howman-Giles (Sydney)	Page 45
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Welcome & Introduction by:

French Ambassador to Australia, H.E. Pierre Viaux

Professor Whitworth,
Professor Garnett,
Professor Syrota,
Distinguished representatives of Australian and French
firms,
Distinguished representatives of universities, research
organisations and associations,
Ladies and gentlemen,

Ten years ago, in 1992, ANSTO and the French Atomic Energy Commission signed an agreement to develop cooperation on the peaceful uses of advanced nuclear technologies. This enabled projects to develop with particular interest on both sides, and the agreement was renewed in September 1997 for another 5 years. The first subject field in which major collaboration was established was Nuclear Medicine.

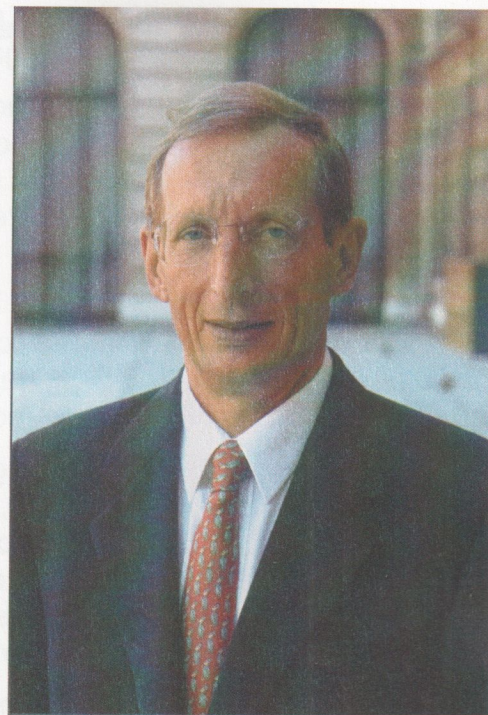
On the French side, this collaboration has been (and still is) inspired and led by Professor Bernard Mazière, whom many of you know, and who is here with us today. On the Australian side, Professor Helen Garnett, who together with Dr. D. Cook hosted the first Australian-French Symposium on Nuclear Medicine in 1992, has also been personally involved in this cooperation throughout this time. I would like to thank Prof Garnett and Prof Mazière personally, and through them all their colleagues, for their continuous involvement.

This continuity is typical of the many people who became involved as the work progressed, allowing our cooperation to grow on a solid basis. One of these people, who is present today and whom I salute, is Professor Gerhard Lowenthal. His contribution to nuclear medicine has been recognised by our two countries, by the awards of the Ordre National du Mérite and the Order of Australia.

You have come here today from a number of Australian and French universities, research organisations and hospitals. You show that in ten years we have established a strong network in a scientifically focused and socially important field.

During these ten years, several major events and achievements have taken place. I will highlight only three of the recent events, each very different in nature.

In March 2000, two conferences on French developments in Nuclear Medicine were held in Melbourne and Sydney. They attracted more than 80 distinguished Australian professionals. They were organized by the Australian-French AFAS associations in New South Wales and Victoria, AFAS being the Australian French Association of Science. Since then, a new President, Dr Carl Munoz-Ferrada, who is with us today, has lead AFAS New South Wales. He has made a strong contribution to the subject,



being the President of Gammasonics, he is also a specialist in nuclear medicine. AFAS receives significant support from the French Embassy.

In May 2000 in Nantes and in December 2000 in Sydney, two seminars focusing on special projects were organised within the framework of FAIR.

FAIR stands for the French-Australian Industrial Research program, which facilitates the establishment and support of links between the industries and research teams of our two countries. The FAIR seminars marked stages in the collaboration between ANSTO, Murdoch University in Perth, Fremantle Hospital, and the French Institute for Medical Research INSERM.

They led to the design of new radio-pharmaceuticals and the creation of a start-up company, Chelatec, which is represented here today. In Australia, most aspects of the FAIR program are managed by the Australian Department of Education, Science and Training and by the French Embassy. They are both sponsors of this Symposium.

In October 2001, a delegation from the University of Tours signed an agreement with the University of Sydney, which will allow us to start double-badged PhDs. It is planned that two PhD students, one from each country, will initially take part in this scheme, in the field of Radiopharmacology. This will further strengthen the cooperation between the teams of Professor Denis Guilloteau and Professor Macdonald Christie, and provide the students with a rich international experience.

Today's symposium is the culmination of these earlier initiatives and of many others. It aims to bring these initiatives together, and be a starting point for establishing a network linking researchers, students and private companies. We already have such a structure in place for hosting this network, with FEAST, the Forum for European-Australian Science and Technology cooperation, and more specifically with FEAST-France, its France-Australia sub-network. You are indeed welcome to join FEAST-France and even, if you wish, create a Special Interest Group on Nuclear Medicine. My colleague Alain Moulet, Science and Technology Attaché, will be glad to answer any questions about these networks.

To conclude, I wish to thank Professor Whitworth for supporting the French-Australian relationship by opening this Symposium with me. I also thank Professor Syrota, Director of Life Sciences at the Atomic Energy Commission, for agreeing to give the plenary lecture.

And I give special thanks to ANSTO for organising this Symposium and making it possible.

I am sure that France and ANSTO can strengthen their relationship further still. As you know, nuclear medicine originated in France with the Curie family. Furthermore, nearly 80% of the electrical energy in France is provided by nuclear reactors. Therefore, we feel that we have an outstanding day-to-day security track record, and that our experience can be usefully shared.

Thank you.

Welcome & Introduction by:

**Director of the John Curtin School of Medical Research,
Australian National University,
Professor Judith Whitworth**

M Moulet, Professor Helen Garnett, distinguished guests,
colleagues

My first scientific hero, when I was at primary school, was a Frenchwoman by marriage, Marie Curie. She overcame crippling poverty and the early and tragic death of her husband and colleague Pierre, to become one of the world's greatest scientists. She was the first person to become a double Nobel Laureate. It was her work, over a century ago in Paris, that gave birth to the concept of radioactivity, and ultimately to the discipline of Nuclear Medicine we are celebrating today.

When I worked and studied in Paris, in the early seventies, supported both by the College of Physicians here and the Hopitaux de Paris, relations between our two countries were strained by another sort of nuclear activity. Because of French nuclear testing in the Pacific, Australian unions had imposed a series of bans and there was not even mail between the two countries.. That notwithstanding, my colleagues were hospitable, and people in general very well disposed to Australians. They remembered Australians fighting in France in WWI and remembered that we had many values in common.

I was enormously impressed by French culture and civilisation, by the very high quality of medical science, and by the breadth of education.

A number of my colleagues had similar experiences and established links with French doctors and scientists, working both in France and in Australia. A few of the working collaborations led not only to publications but to marriages and off spring. Through NHMRC and INSERM we set up a joint cardiovascular collaboration that has been meeting over more than a decade. There are many similar initiatives.

Today, of course, apart from the occasional punch-up on the rugby field, our two countries are again very close and today is a scientific celebration of that closeness. The cross fertilisation of scientific and technical skills is evident in today's program.

A major presentation today discusses a French Benzamide labelled with I-123 that it is hoped will show up better in Malignant melanoma — the Australian disease — than the current gold standard marker FDG. A clinical trial in Sydney is about to start. In parallel, will be ongoing research to make a therapy analogue with I-131, to actually treat widespread deposits of this particularly vicious disease.



In broad terms Nuclear Medicine has swept across medical science from therapy to imaging and back, but on the way has 'spun off' specialised sub-groups that concentrate on particular organs, for example 'Nuclear Cardiology', or particular technologies such as Positron Emission Tomography. In more recent times a good deal of research focus has been on assisting with new drug development through the exquisite sensitivity of the radio-tracer principle. By labelling new chemical entities and injecting them into test animals it is possible today to quantitate directly the uptake in specific organs or disease states in vivo.

The meeting today covers almost the entire spectrum of applications of radionuclides in clinical medicine and drug development.

Apart from being at the forefront in using the well-established measuring tools involving in vitro identification of bio-molecules, my own institution, the John Curtin School of Medical Research at the Australian National University in Canberra, has a long history of direct involvement with clinical Nuclear Medicine. We were part of a multi-disciplinary team that made Thallium-201 for heart imaging on the ANU cyclotron in 1976. We were at the start of a lung ventilation research program whose output is now used in 35 countries with over 1 million patients studied. Bill Burch is one of our distinguished Visiting Fellows. We have worked with ANSTO on tumour visualisation with long term possibilities for tumour diagnoses and therapy, and we look forward to similar collaborations in days to come.

I wish you all *bonne chance* for a productive and exciting meeting.

PLENARY INVITED LECTURE:

Functional imaging with radionuclides: a vital tool for drug development and cell and gene therapy

**Professor André Syrota, Director Health Sciences,
French Atomic Energy Commission (CEA), Orsay, France
(andre.syrota@cea.fr)**

The conference reviews some important contributions of PET to the drug development process such as the pharmacokinetics studies in normal volunteers or in patients, of the demonstration of the mechanism of action via the modification of blood flow/metabolism or via the interaction with an enzyme, a receptor, an ion channel, a transporter, a mRNA. Finally, some up-to-date examples of PET for assessing the effect of gene or cell therapy are described.

PET in the drug development process can be used with profit in three different situations:

1. when the drug itself has been labelled with a positron emitter, the tissular pharmacokinetics of the drug and its in vivo pharmacological properties such as its binding characteristics on a receptor, a transporter or an enzyme can be assessed;
2. when the drug has not yet been labelled, PET allows to assess the interaction of the cold drug with a radiolabelled probe specific of a neurotransmission phenomenon or with a radiolabelled substrate involved in specific biochemical process;
3. finally, PET can be used to image new therapies such as the gene or cell therapies.

The unique benefits of these different and complementary applications of functional imaging with radioisotopes are illustrated with recent examples.

The remarkable interest of PET for pharmacokinetics studies is highlighted in the following cases related to traditional drugs and to a new class of potential drugs, the antisense oligonucleotides:

- Befloxatone, an oxazolidinone derivative, is a new selective MAO-A reversible inhibitor with potential antidepressant properties. Befloxatone has been labelled with ^{11}C and the PET study in baboon and in man has provided unique information on the brain pharmacokinetics profile of befloxatone (level and time course of MAO-A inhibition). Moreover, from the conclusions of this study, ^{11}C -befloxatone proves to be an excellent "probe" for investigating the pathological and pharmacological modifications of MAO-A activity in man.
- In the same way, 18F- A-85 380, a nicotinic acetylcholine receptor antagonist, has been validated as a radiolabelled probe and is now available for investigating in human drug dosage in psychiatry.
- Oligonucleotides have been proposed, for therapeutic purposes, to block the expression of a gene. Recently it has been shown that it is possible to label an oligonucleotide with a positron emitter, essentially ^{18}F , either in the 3' or 5' position. Despite the many problems existing in antisense imaging (rapid degradation of oligos in plasma and tissues, binding to plasma proteins, low penetration into cells, binding affinity to mRNA, radiotoxicity of labelled antisense oligonucleotides, ...)

chemical modifications of their structures make them stable enough to be used for PET in vivo kinetics studies in baboon.

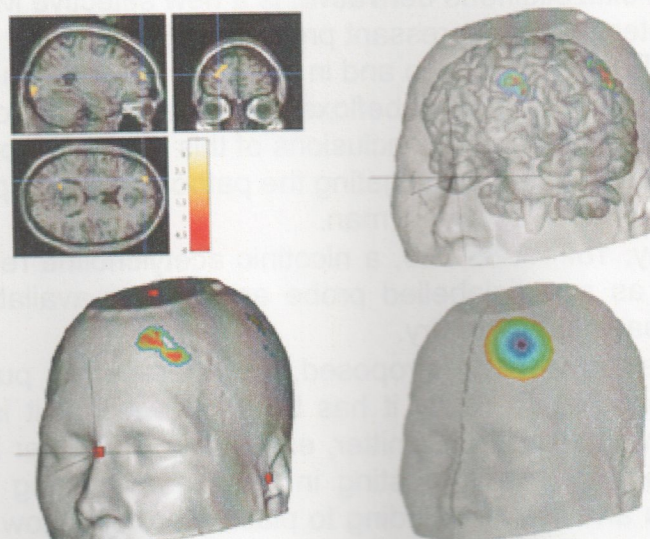
Examples related to the pharmacology of Schizophrenia exemplified the scientific and clinical benefits of PET of brain receptor imaging. In schizophrenia research, the dopaminergic system has received greatest attention and a linear correlation has been demonstrated between antipsychotic drug affinity for D2 receptors in animal brains in vitro and clinical affinity potency in man. Moreover, the adverse effects, like the extrapyramidal symptoms are known to be correlated with high in vivo occupancy of striatal D2 receptors. Optimising the posology by rapidly determining the doses that occupy the optimal percentage of receptors (over 70% for being effective and under 80% to avoid negative side effects) is then a major therapeutic problem.

Using D2 receptor radioligands with high affinity ($[^{11}\text{C}]$ -Raclopride, $[^{76}\text{Br}]$ -bromo spiperone, $[^{76}\text{Br}]$ -BLIS) or with very high affinity ($[^{11}\text{C}]$, $[^{76}\text{Br}]$ -FLB 457, $[^{11}\text{C}]$ -Epidepride) it is now possible to quantify the D2 receptor density in striatal and extrastriatal brain structures. In vivo PET investigations performed using these probes under different therapeutic antipsychotic regimens are particularly helpful in determining the occupancy profiles of new atypical antipsychotics (clozapine, olanzapine, amisulpride).

Moreover, radionuclide functional imaging allows to directly or indirectly investigate the pathological alteration and the possible pharmacological restoration of a given biochemical process such as blood flow or glucose metabolism.

- Thus, in Alzheimer's disease patients, PET investigations have demonstrated a significant decreased blood flow in the temporo-occipital cortex but also a smaller decrease in the frontal cortex while in autistic children bi-temporal hypoperfusion has been characterised.
- In depressed patients in vivo assessment of glucose energy metabolism using ^{18}F -FDG allows to accurately localise the brain regions with a significantly decreased metabolism. These very same regions appear to be specifically stimulated after classical pharmacological antidepressant treatments or up-to-date transcranial magnetic stimulations.

FDG PET images in Depression - application to TMS



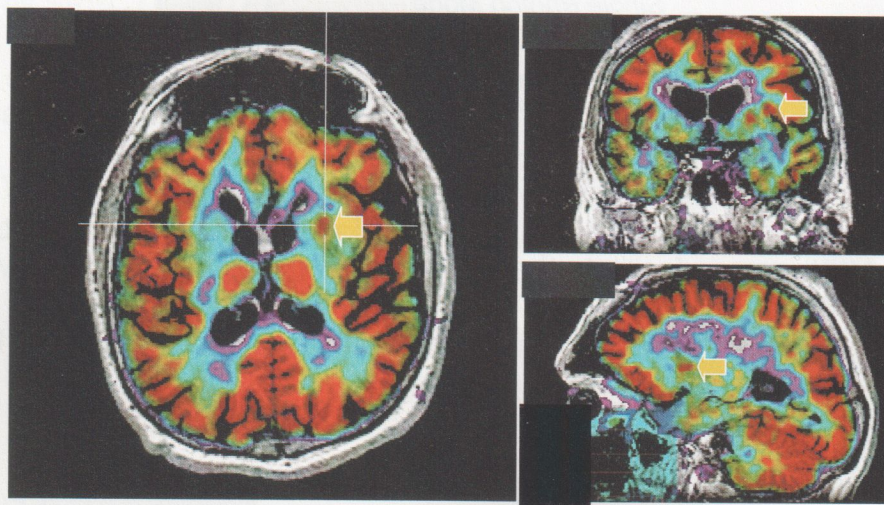
Finally, the unique opportunities offered by PET for imaging new therapies such as foetal cell grafting, gene expression and gene therapy, are brought forward.

- ^{18}F -FDOPA is routinely used in clinics to evaluate the severity of neurodegenerative diseases such as Parkinson or Huntington diseases. In clinical investigation, the same tracer allows to visualise and evaluate the viability of nigral or striatal neuronal grafts used as a substitutive therapeutic and also to investigate the efficacy of specific drugs or neuroprotection factors.

- Visualising gene expression in animal is now possible using radiolabelled oligo nucleotides or the reporter gene/radiolabelled reporter probe system.

In oncology, antiviral drugs, mainly guanine derivatives, such as ganciclovir or penciclovir, are experimented as pro-drugs. Using the viral enzyme expressed by the gene of viral thymidine kinase (tk) introduced in the dividing metastatic cells with the help of a convenient viral construction, the prodrug is transformed in the phosphorylated guanine derivative analogue, which is the really effective cytotoxic drug. For this application, the efficacy of the transfection is a key issue. To visualise this important parameter, a radiolabelled analogue, i.e. fluoro-ganciclovir labelled with F-18, can be used as a probe. The ^{18}F -guanine derivative which is also phosphorylated by the viral Tk will accumulate in the tumour cells according to the

Neurotransplantation in Huntington's Disease



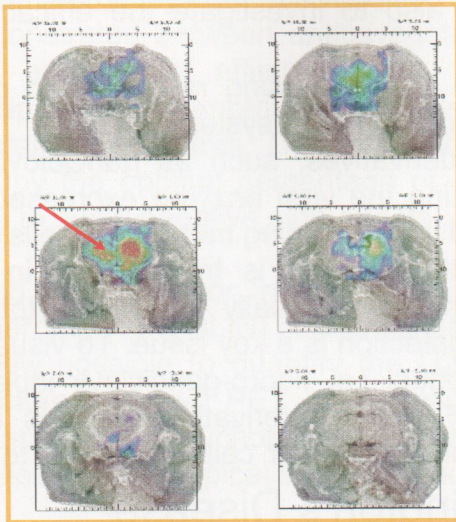
^{18}F -fluorodeoxyglucose PET scans (2 years Post-graft)

density of the expressed enzyme allowing to evaluate the efficacy of the treatment.

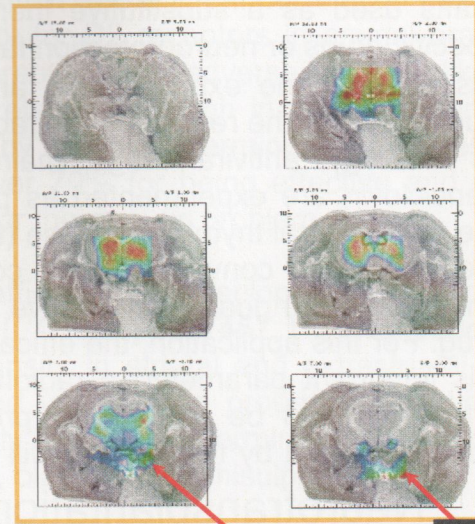
In a near future, small animal in imaging techniques should play a substantial role to monitor gene therapy. State-of-the-art small animal in vivo imaging PET and SPECT systems (i.e. THOR) are reviewed and some striking applications described.

TOHR : High Resolution γ Tomograph for small animals

^{123}I -Epidepride $\sim 500 \mu\text{Ci}$
neurodegeneration



^{123}I -PE2I $\sim 500 \mu\text{Ci}$
localization of substantia nigra



Single Photon Mode

SN

SN

It is then nowadays admitted that radionuclide functional imaging and more particularly PET provides unique information all the way down the line of the three classical phases of the development of new drugs :

- during phase I, PET allows to assess in vivo regional kinetics of the drugs in human;
- during phase II, PET allows the accurate measurement of specific binding site occupancy and then can facilitate the evaluation of the "optimal" doses which will be used in phase III;
- and during phase III, PET can provide a surrogate end-point to evaluate the efficacy of the drug.

SESSION 1

"State of the art in both in vitro and in vivo aspects of small animal imaging"

B. Mazière (SHFJ, Orsay, CEA) (maziere@dsv.cea.fr)

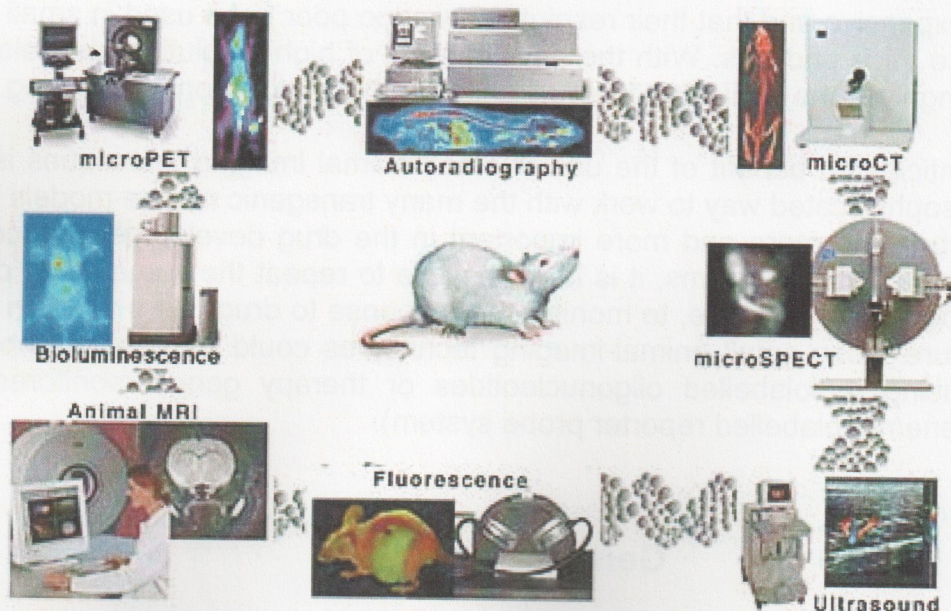
D. Guilloteau (Université de Tours) (denis.guilloteau@med.univ-tours.fr)

D. Lebars (CERMEP, Lyon) (lebars@univ-lyon1.fr)

In vivo imaging for small animals is dramatically expanding due to the coincidence of mainly three technical factors:

- the explosion in computer power
- the enhancement in image processing
- the accessibility and affordability of digital autoradiography systems and small-animal scanners

Among these imaging techniques let us mention the anatomical imaging techniques such as ultrasonography, X-rays and IRM and the functional imaging radioisotopic techniques SPECT and TEP.



The main advantage of the first group of imaging techniques is essentially linked to the high resolution of the anatomical images (with the drawback of the necessity of putting the animal at rest using anaesthesia).

The main advantages of SPECT and PET are their high sensitivity and the vast number of functions or metabolism they allow to image.

The applications for isotopic functional imaging in small animals are increasing rapidly. Factors contributing to this dramatic expansion include the three previous technical factors plus, at least, three methodological factors:

- the drug discovery process based on receptor / mechanism of action
- the increasing number of rodent models of human diseases (SCID mice implanted with human tumors, gene knock-out mice, transgene mice, ...)
- the advances in isotope and validated tracer availability performances

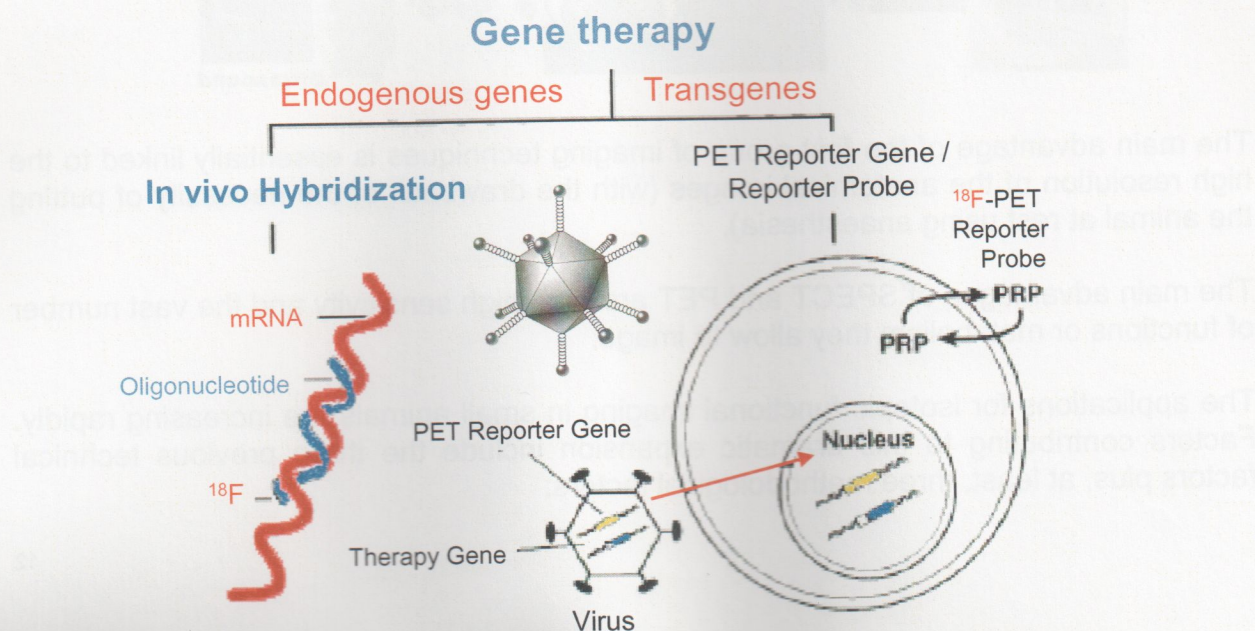
Small animal radioisotopic functional imaging for drug development.

In vivo quantification of biological processes to measure the mechanism of action of a potential drug and its concentration at the site of action has become mandatory for developing a drug.

Rational and efficient means of confirming mechanisms of action are required. For this purpose, PET and/or SPECT functional - biochemical - molecular imaging in small animals are tools of choice for economical reasons (in the domain of drug development, industry is suffering huge opportunity costs by failing to weed out non-performing new active substances until late phases II and III) and for practical reasons (with the advent of genomics and combinatorial chemistry, gene knock-out mice, transgene mice and cloned human receptors, drug discovery departments could be rapidly flooded with new active compounds making the selection for further development becoming more and more critical).

Until recently, it was felt that PET and SPECT radioisotopic imaging methodologies were too expensive and that their resolution was too poor to be used in small laboratory animals like mice and rats. With the development of high-resolution and relatively low-cost imaging systems dedicated to small animals, that perception is changing.

Another anticipated benefit of the use of small animal imaging techniques is that they provide a sophisticated way to work with the many transgenic mouse models of disease that have become more and more important in the drug development process. Using these in vivo imaging systems, it is now possible to repeat the same study on a single mouse allowing, for example, to monitor the response to drugs as a function of time. In a near future these small animal-imaging techniques could be used to monitor gene therapy (using radiolabelled oligonucleotides or therapy genes monitored with the reporter gene/radiolabelled reporter probe system).



Conclusion :

Animal PET/SPECT systems are unique tools to provide information on the mechanism of action of drugs and as such are more and more used as rapid screening procedures for selecting new drugs and for optimising treatment protocols.

The use of small animal imaging systems is particularly valuable in small animal studies with a longitudinal or interventional design.

The high-performance, application-specific research PET and SPECT systems, designed for use with small animals, should facilitate quantitative accurate research data to be translated more effectively from pharmacological labs to clinical medicine and clinical trials.

PET and SPECT for small-animal imaging provide a bridge between preclinical drug development scientists and clinicians running phase I to III trials.

“Recent Developments in Instrumentation for Pre-Clinical Imaging Studies”

Steven R. Meikle, Ph.D. (steve@nucmed.rpa.cs.nsw.gov.au)

**Department of PET and Nuclear Medicine
Royal Prince Alfred Hospital, Sydney, Australia**

Abstract

Recent advances in imaging instrumentation have led to a variety of tomograph designs for dedicated pre clinical imaging of laboratory animals. These advances make it possible to image and quantify the kinetics of radiolabelled pharmaceuticals in a wide range of animal models from rodents to non-human primates. Applications include evaluation of promising new radiopharmaceuticals, study of the molecular origins of human disease and evaluation of new forms of therapy. These applications and advances in instrumentation are equally applicable to positron emitters and single photon emitters. This paper provides an overview of recent advances which have led to the current state-of-the-art in pre clinical imaging.

New Inorganic Scintillators

Table 1 lists the common inorganic scintillators that have been used for SPECT and PET, including some of the promising materials recently studied. The current crystal of choice for SPECT imaging is NaI(Tl) because of its high light output and density which make it well suited to imaging photons in the 100-200 keV range. However, NaI(Tl) has the disadvantage that it must be hermetically sealed to prevent absorption of moisture from the environment. Therefore, investigators have explored a number of alternative inorganic crystals, including CsI(Tl) and cerium-doped yttrium aluminium perovskite (YAP), as well as solid state detectors such as cadmium zinc telluride (CZT). Many of the crystals used in SPECT have also been tried for PET, including NaI(Tl) and YAP. However these crystals have lower stopping power than BGO and NaI(Tl) is also relatively slow. A very promising scintillator for PET is cerium-doped lutetium oxyorthosilicate (LSO) (1) which has similar stopping power to BGO and relatively high light output and fast decay. The first PET scanner to use LSO was the UCLA animal scanner, microPET (2, 3) (Figure 1), which also makes use of a number of other new technologies and unique design features.

Table 1. Properties of Common and Promising New Scintillators for SPECT and PET

Scintillator	ρ (g/cc)	Z _{eff}	Yield (%)	τ (ns)	λ (nm)	Hygroscopic?
NaI(Tl)	3.67	51	100	230	410	yes
CsI(Tl)	4.51	54	45	1000	565	slightly
CsI(Na)	4.51	54	75	630	420	yes
Y ₂ SiO ₅ (Ce) (YSO)	4.54	34	118	70	420	no
YAlO ₃ (Ce) (YAP)	5.37	39	40	25	370	no
Gd ₂ SiO ₅ (Ce) (GSO)	6.71	59	30	60, 600	430	no
Bi ₄ Ge ₃ O ₁₂ (BGO)	7.13	75	15	300	480	no
Lu ₂ SiO ₅ (Ce) (LSO)	7.40	65	75	40	420	no
LuAlO ₃ (Ce) (LuAP)	8.30	53	50	18	365	no

Advances in Photodetectors

Recently, improvements in multi-anode and crossed wire position sensitive photomultiplier tubes (PS-PMT) have enabled a single photodetector to decode signals produced in a crystal slab or array of small crystals. While these devices are relatively bulky and include a large dead area at the periphery, a number of investigators have been able to make use of their high intrinsic spatial resolution in small animal detectors. For example, the microPET scanner uses optical fibres to couple PS-PMTs indirectly to LSO crystal arrays which overcomes the dead space problem (4) (Figure 1b). Similarly, at RPAH we are developing the CoALA-SPECT camera with detectors based on a PS-PMT, pixellated NaI(Tl) crystal and multi-pinhole aperture (5) (Figure 2). Solid state photodetectors, including silicon photodiodes and avalanche photodiodes (APD), provide promising alternatives to PMTs. It is possible to achieve one-to-one coupling of these devices to small scintillator crystals (2-3 mm wide), as seen in the small animal PET system developed at the University of Munich (6).

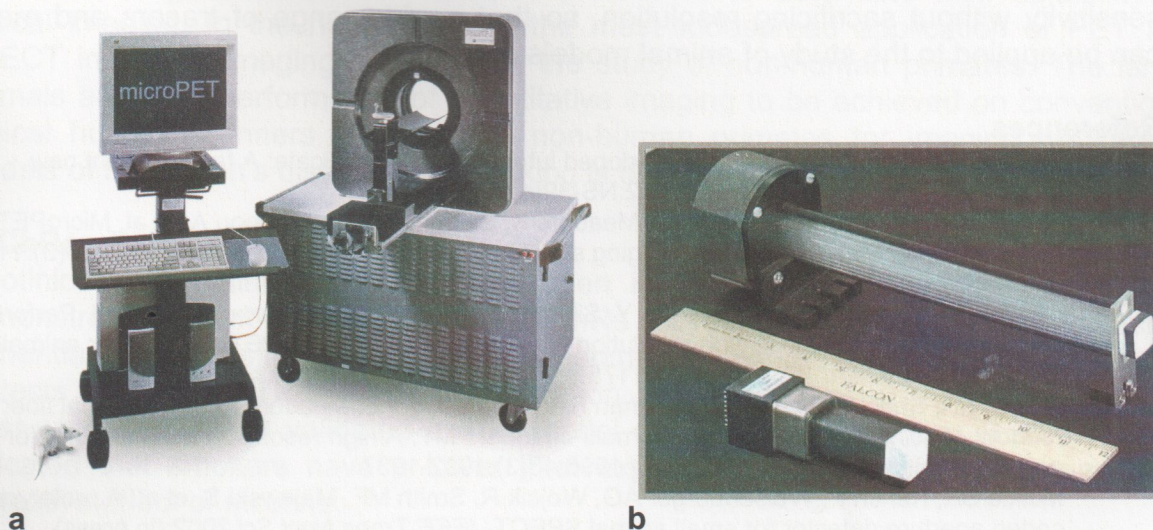


Fig. 1: (a) microPET animal scanner and (b) detector modules for the microPET research prototype (background) and commercial prototype (foreground), showing PS-PMT, LSO crystal array and optical fibre light guides.

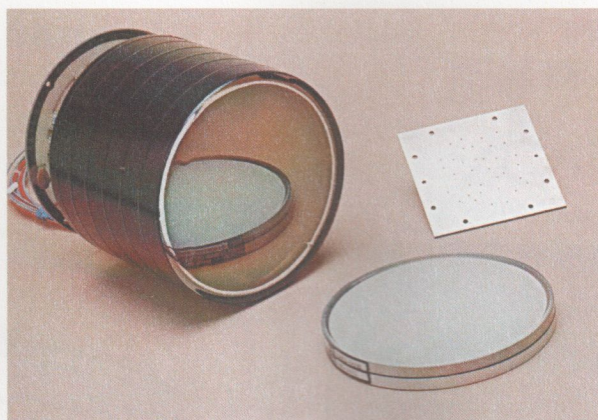


Fig 2: CoALA-SPECT detector components, showing pixellated NaI(Tl) crystal array and light guide (foreground), PS-PMT (back left) and multi-pinhole aperture (back right).

Summary

A number of promising new detector technologies have been developed and refined over the past 5-10 years. These include:

- New scintillators such as LSO and YAP
- Position sensitive PMTs
- Photodiode arrays
- Fibre optic light guides

The current state-of-the-art in small animal PET and SPECT scanners employ one or more of these technologies to achieve isotropic spatial resolution approaching 1 mm full width at half maximum (FWHM). Further advances are likely to see the spatial resolution of these systems fall below 1 mm FWHM which will challenge the fundamental limit of resolution that can be achieved with PET due to positron range and non-collinearity. The challenge in the design of such systems will be to improve sensitivity without sacrificing resolution, so that a wide range of tracers and methods can be applied to the study of animal models of disease.

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"Studies of Nicotinic Receptors in Non-human Primates Using PET and SPECT"

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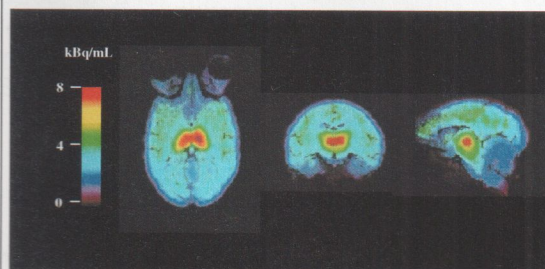
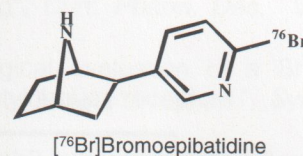
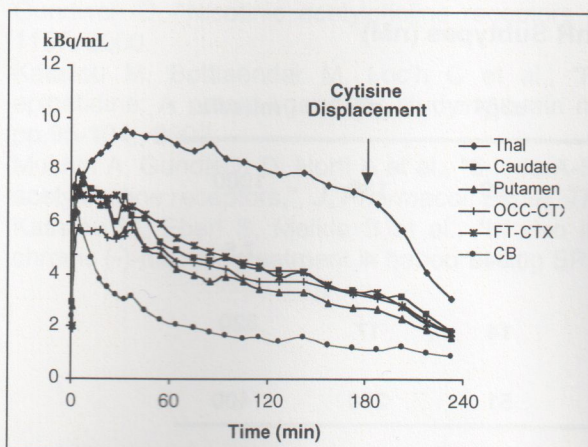
**Department of PET and Nuclear Medicine, Royal Prince Alfred Hospital,
Department of Pharmacology, University of Sydney**

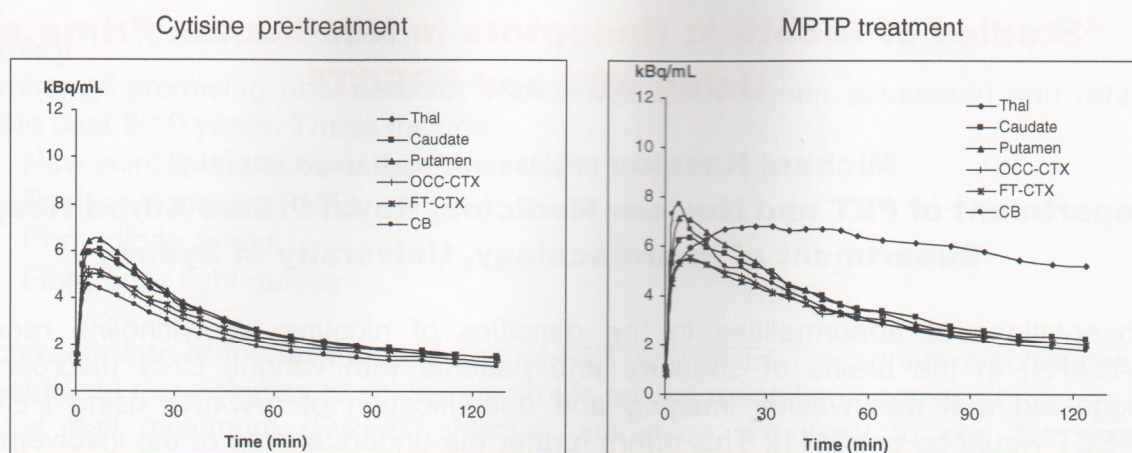
Observations of abnormalities in the densities of nicotinic acetylcholine receptors (nAChRs) in the brains of smokers and patients with various CNS disorders has suggested that noninvasive imaging and quantification of nAChRs using PET and SPECT would be useful [1]. This offers further the understanding of the involvement of these receptors in these conditions as well as insight into their role in the normal functioning of the brain. As a prelude to human studies, newly developed PET and SPECT radioligands are first evaluated in animals to determine their suitability for clinical imaging. In the neurosciences the most widespread application of PET and SPECT in animal imaging has been in the study of non-human primates. The larger animals allow the performance of quantitative imaging to be achieved on conventional clinical human scanners. The use of non-human primates for imaging nAChRs in models of Parkinson's disease and smoking is described below.

nAChRs - Parkinson's disease (PD)

Nicotinic acetylcholine receptors have been implicated in PD's since it has been demonstrated postmortem that cortical nAChRs are reduced and parallel the increase in dementia that occurs in PD patients. In experimental animals it has shown that nicotine protects against MPTP-induced degeneration. MPTP degeneration representing the most widely used and validated animal model of PD. Also, a number of studies have indicated that smokers have a lower than expected incidence of PD, suggesting a protective effect of nicotine.

In order to study nAChRs using PET we have developed [^{76}Br]bromoepibatidine. This work was carried out at the Service Hospitalier Frederic Joliot, Orsay France as part of the France-Australia collaboration. In papio papio baboon the brain uptake of [^{76}Br]bromoepibatidine was high with preferential localisation in the thalamus. The [^{76}Br]bromoepibatidine uptake is consistent with the known cerebral nAChR distribution in primates.

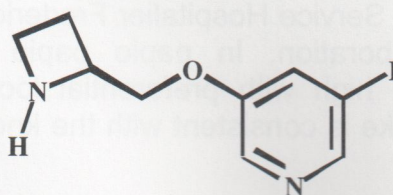




The radioactivity in thalamus, striatum and cortices was reduced to the level of the cerebellum in both displacement and pre-treatment experiments using cytisine. This clearly demonstrates the specificity and saturability of [^{76}Br]bromoepibatidine binding to nAChRs. In a baboon that had been treated with MPTP the uptake of [^{76}Br]bromoepibatidine, when compared to the control animal, was reduced in the thalamus by 50% while the radioactivity in the striatum and cortices was similar to that of the cerebellum. These results suggest that [^{76}Br]bromoepibatidine has the potential to be a useful radioligand for studying the pharmacology of nAChRs [2].

nAChRs - Smoking

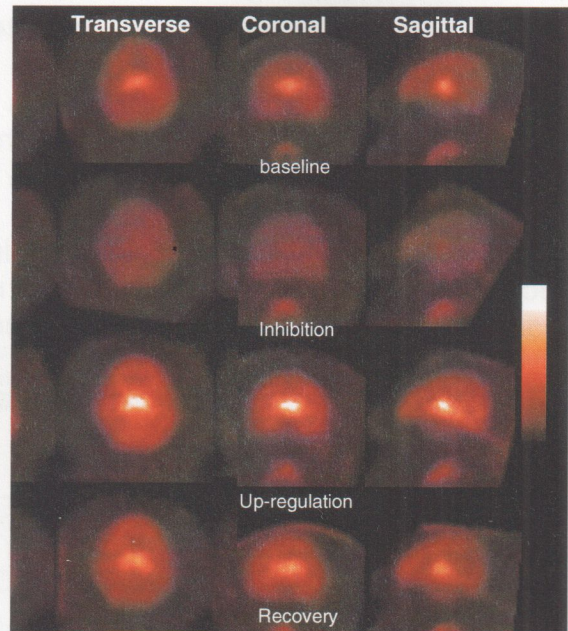
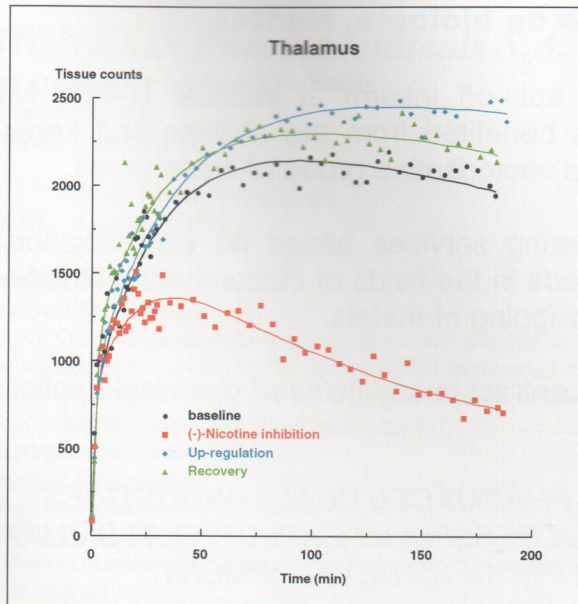
Nicotinic acetylcholine receptors are shown to be increased in cortex, cerebellum, hippocampus and thalamus homogenates of autopsied brain samples from smokers. A positive correlation between cigarette consumption and nAChR increase has been identified, indicating dose dependent increase. Smokers who had stopped smoking at least 2 months before death had nAChR levels similar to those of non-smokers. In an attempt to visualise and quantify in vivo nAChR upregulation the subtype selective radioligand 5-[^{123}I]iodo-A85380 [3] was used with SPECT and kinetic modelling.



Ki at nAChR Subtypes (nM)

Ligand	$\alpha 4\beta 2$	$\alpha 3\beta 4$	$\alpha 7$	muscle
(-)-nicotine	0.84	100	130	1000
($\pm$)epibatidine	0.008	0.049	4	7.5
A-85380	0.017	14	17	320
5-iodo-A-85380	0.01	51	250	1400

In order to induce nAChR upregulation a male papio hamadryas baboon was chronically treated with nicotine (2 mg/kg/24h) for 14 days by subcutaneous implantation of an osmotic pump. Blood samples were taken during the dosing period to determine the amount of nicotine in plasma.



A SPECT study was performed prior to nicotine dosing to obtain baseline levels of 5- ^{123}I iodo-A85380 uptake. When imaging was performed while nicotine was still found in plasma resulted in an inhibition study. Seven days following removal of the osmotic pump resulted in increased 5- ^{123}I iodo-A85380 in thalamus, cortex and cerebellum, while images obtained 4 weeks later indicated some recovery and levels returning back close to baseline [4]. This clearly demonstrates in vivo nAChR upregulation with 5- ^{123}I iodo-A85380 and quantitative dynamic SPECT.

Although the development of new imaging probes and advances in molecular biology has increased the use of rats and mice, imaging studies of non-human primate CNS will continue to play an important role in the evaluation of new radioligands and pharmacological evaluation of diseased states.

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"From academic research to the creation of Chelatec company"

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CHELATEC was founded in 2000 as a spin-off inserm at institute U-463, Nantes Hospital, France. The present team has benefited from the abilities and know-how acquired in radioimmunotargeting within the vectorisation group of inserm.

The company provides complete engineering services based on complexation and vectorisation, as adapted to the client's needs in the fields of customised radiolabelling, radioimmunotargeting, decorporation and trapping of metals.

CHELATEC conducts R&D activity under contract in key fields of chemical applications to medicine and environment.

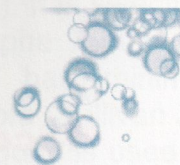
OUR MISSION IS TO ENABLE PHARMACEUTICAL INDUSTRIES AND BIOTECHNOLOGIES COMPANIES TO SUCCESSFULLY EXPLOIT OUR SKILLS AND TO HELP THEM IN THEIR PRODUCT OR PROCESS DEVELOPMENT.

CHELATEC produces and supplies radiolabelled compounds.

For further information about our activities consult our website:

www.chelatec.fr

B I O N E X I S



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Business Description

BIONEXIS Pharmaceuticals is a drug discovery oriented company generating novel molecules designed to recognize reactive lipids abnormally exposed on the surface of cell membranes in cancers, inflammation and cardiovascular diseases. BIONEXIS is focused on drug discovery and development of novel molecules that can be used as therapeutic tools consisting in drugs or specific delivery systems for drugs and/or markers.

Management Team

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Françoise Russo-Marie, BIONEXIS founder and CEO is a former Director of Research INSERM and former chairman of the INSERM Unit 332 at the Institute Cochin, Paris, France. She is an expert in the role of signalling lipids and their interacting proteins in diseases such as cancers, inflammation and cardiovascular diseases.

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Alain Samson, PhD, BIONEXIS founder and CSO, is a former Professor at the University of Pierre et Marie Curie (Paris VI). Prior founding BIONEXIS, he was group leader at CEA (Commissariat à l'Energie Atomique, Saclay, France) studying the structure, folding and molecular modelling of proteins interacting with membrane lipids.

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Jean-Paul Comin, BIONEXIS founder and COO, graduated from ENSAM (Ecole Nationale Supérieure des Arts et Métiers). Prior founding and joining BIONEXIS, he was project manager for missiles at EADS (European Aeronautic Defence and Space Company).

Technology

The technology developed by BIONEXIS results from an innovative approach based on the study of protein-lipid interactions occurring on the surfaces of cell membranes in order to design "intelligent molecules". An emerging field of research concerns diseases associated with lipids abnormally exposed on the surface of cell membranes. Once exposed, host proteins recognize these lipids which initiate biological responses that are often associated with diseases. BIONEXIS is able to identify the domain of the protein engaged in the interaction and to reconstruct this domain in 3D using molecular modeling and bio-informatics. Using these tools, functional peptides or chemical molecules are designed and engineered to recognize the targeted lipid. These molecules, currently developed, will be used as, either drugs to inhibit the biological responses such as thrombosis, inflammation, proliferation or, delivery systems for drugs (drug vectors) or tracers (medical imaging) to target cells exposing abnormal reactive lipids.

Products

Using its technology, BIONEXIS has already constructed and developed its first novel molecule. AFIM first derivatives are the first engineered markers for apoptosis and thrombosis for SPECT, PET and MRI imaging.

Business Activity

- BIONEXIS will be present in three rapidly expanding fields of pharmaceutical innovation: i) anti-thrombosis agents (\$6.7 billion in 1999), ii) drug targeting agents and iii) targeted contrast agents for medical imaging (\$3.5 billion in 1999).
- BIONEXIS's target market is pharmaceutical companies. Thus, the business model of BIONEXIS is to offer innovative products and develop them up to phase IIA clinical trials. The products will then be licensed to pharmaceutical firms for completing the clinical trials to launch the new drugs on the market.

Contact

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SESSION 2

"Nicotinic Receptor Imaging with F-18 A85380 PET in Alzheimer's Disease and Normal Ageing"

A research collaboration between:

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**Department of Nuclear Medicine
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Austin and Repatriation Medical
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Sabina Pappata
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**Christopher Rowe (christopher.rowe@armc.org)
Henri Tochon-Danguy (htd@austin.unimelb.edu.au)
David Reutens
Gordon Chan
Michael Woodward**

Background:

Central nicotinic acetylcholine receptors (nAChR) mediate excitatory neurotransmission and contribute to a variety of brain functions including learning and memory. Post mortem studies in patients with Alzheimer's disease have revealed losses of nAChR from the neocortex and hippocampal formation with ligand binding studies showing a reduction of over 50% compared to normal elderly brains in the temporal cortex and hippocampus (Sabbagh 1998). This is consistent with the loss of cholinergic neurones that has been well documented in this condition. Nicotinic AChR are predominantly located presynaptically on the cholinergic neurones. Consequently the ability to image and quantify these receptors may provide a measure of cholinergic loss and therefore a test for the early diagnosis of Alzheimer's disease and for monitoring therapy designed to preserve cholinergic neurones. Aging is known to effect nAChR (Hellstrom-Lindahl 2000) so this variable must be quantified and incorporated into analysis of the scans.

Nicotinic receptors also have important modulatory effects on glutamate, dopamine, serotonin and noradrenaline release and profound receptor loss has been documented in Parkinson's disease and Diffuse Lewy Body disease in addition to AD. Abnormalities in the alpha 7 subtype have been reported in schizophrenia.

Imaging studies of nAChR have been hampered by the lack of a suitable tracer for in-vivo imaging. Nicotine itself labelled with carbon-11 for PET imaging has been used but has been shown to reflect regional cerebral blood flow not nAChR due to high nonspecific binding (Nyback et al, 1994). Potent nAChR ligands such as Epibatidine have been very useful for in-vitro studies but are too toxic for routine human use due to strong activation of nAChR including those in the sympathetic ganglia (A3B4 subtype).

Recently, the Abbott Laboratories developed A85380 (3-[2(S)-2-azetidylmethoxyl]pyridine) an azetidine derivative of the 3-pyridyl ethers that has high

affinity but low potency for the nicotinic receptor (i.e. it is a weak agonist). It is selective for the $\alpha 2\beta 4$ (A2B4) subtype. A85380 does not bind to the $\alpha 7$ subtype receptor nor the ganglionic $\alpha 3\beta 4$ subtype and therefore has minimal cardiovascular effects. It also has low affinity for muscle nicotinic receptors (Sullivan JP 1996). These properties indicate a high level of safety when this compound is used in the extremely low (nanomolar) concentrations required for PET imaging studies. Labelling of A85380 with fluorine-18, a positron emitting radioisotope, has been achieved without alteration of the receptor binding characteristics by Frederic Dolle and colleagues at the CEA PET Centre, Orsay (Dolle F et al., 1998).

In March 2000, F-18 A85380 PET studies in baboons were presented by Prof. Bernard Maziere at the France Australia Scientific Meeting in Melbourne. After this presentation a collaboration between the CEA PET Centre and ARMC was discussed between Prof. Maziere and Dr. Rowe (Director, Department of Nuclear Medicine and Centre for PET, ARMC). Subsequently in June 2000 at the US SNM meeting in St. Louis further discussions were held between Dr. Rowe and Professor Syrota.

In September 2000, draft protocols were exchanged and communication between Frederic Dolle and Henri Tochon-Danguy commenced regarding the radiochemistry. In May 2001, Dr. Rowe visited the CEA PET Centre, Orsay and a collaborative agreement was signed. Subsequently between May and August 2001, CEA PET Centre supplied the chemical precursor, labelling method, toxicology results and human radiation dosimetry data to ARMC.

The research plan is to investigate the uptake and distribution of F-18 A85380 with PET in normal elderly persons, and subjects with mild Alzheimer's disease, and to quantify the effect of age on uptake. We will assess the utility of nicotinic receptor imaging with PET for the early diagnosis of AD and its potential for monitoring therapies designed to preserve cholinergic neurones.

Aims:

To determine the distribution of F-18 A85380 in the normal human brain in persons aged between 50 and 80 years.

To determine the effect of age on F-18 A85380 binding.

To determine the distribution of F-18 A85380 in the brain of persons with mild Alzheimer's disease.

To determine if F-18 A85380 PET can reliably differentiate persons with early Alzheimer's disease from elderly normal persons.

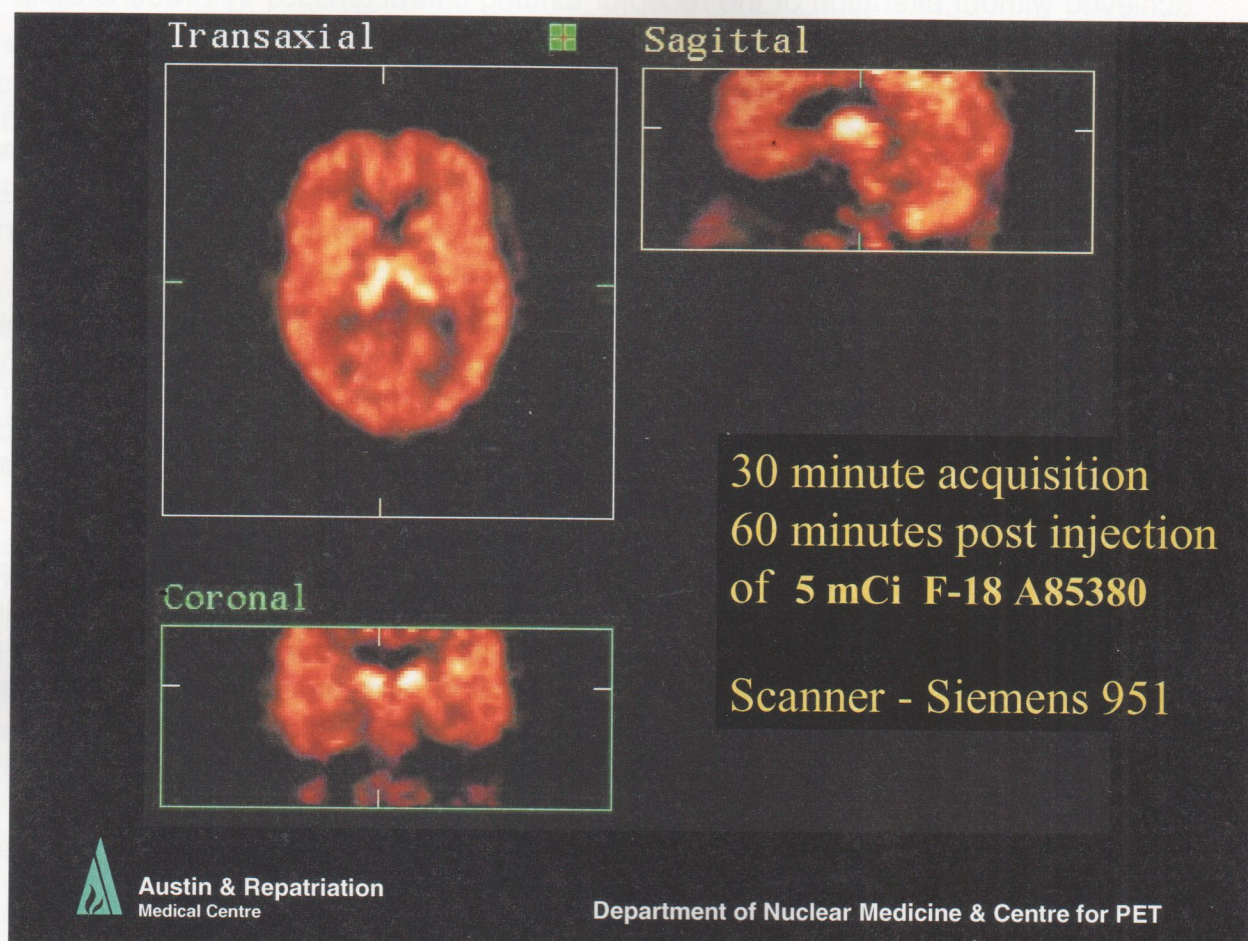
Progress:

In April 2002 the first human study of nicotinic receptors with F-18 A85380 was performed at ARMC. The scan is shown in figure 1. Difficulties with Ethics approval delayed the commencement of scanning in France but human studies have now commenced at both sites. Both Centres are working together to optimise scan times and determine the most appropriate analysis technique.

Both Centres plan to study at least 20 mild AD subjects and at least 20 age matched normal subjects. The data will be pooled for joint publication.

Future studies in Mild Cognitive Impairment, familial frontal lobe epilepsy, schizophrenia, and Parkinson's Disease may follow.

Figure 1



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Development of PET and SPECT Radiopharmaceuticals to Study Multi-Drug Resistance (MDR)

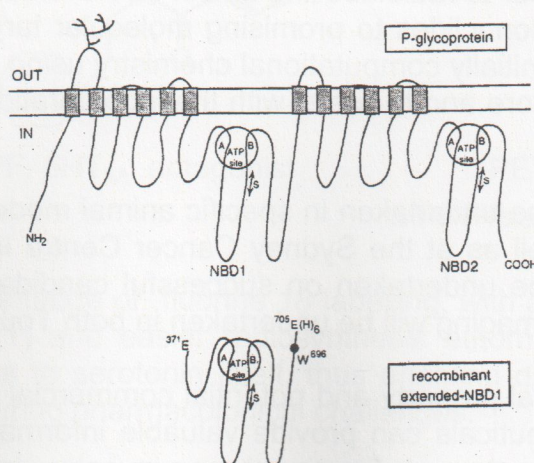
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Cellular resistance or Multidrug Resistance (MDR) to cytotoxic agents is the major cause of treatment failure in many human cancers. P-glycoprotein (Pgp), a Mr 17,000 transmembrane protein and Multi Resistance Protein (MRP) are two proteins that are over expressed and confer resistance to a large number of chemotherapeutic agents by enhancing their extracellular transport. P-glycoprotein is expressed at a relative high level in treated and untreated human malignant tumours, including renal, colonic, adrenal, hepatocellular carcinoma and a considerable percentage of breast carcinomas.

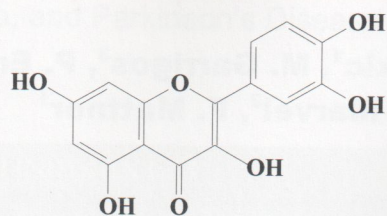
^{99m}Tc-Sestamibi, a lipophilic cationic complex is a transport substrate for Pgp. In clinical studies of human neoplasms it was found that tumour uptake and clearance of this tracer correlate with Pgp expression and may be used for the phenotypic assessment of MDR. However, new tracers with better substrate specificity for Pgp and other drug transporters would greatly assist in optimising chemotherapeutic treatment and improving patient management by predicting tumour response to therapy and to assist in the development of antagonists, which may reverse or halt MDR.



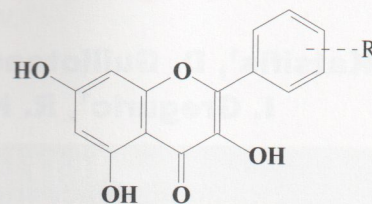
The aim of this project is therefore to develop PET and SPECT radiopharmaceuticals with improved affinity and selectivity for Pgp and MRP for the clinical evaluation of MDR in cancer patients.

To optimise cellular transport characteristics, a number of chemical families that have been found to be substrates of Pgp and other drug efflux pumps, will be investigated. In

the first instance, a series of drugs based on the flavonol natural product, Quercetin will be developed, screened for MDR and radiolabelled with PET and SPECT isotopes.



Quercetin



* Flavonol Derivatives

Quercetin and related flavonol derivatives have been selected for this project because of their moderate to good affinity for Pgp. With the assistance of molecular modeling and *in vitro* studies, structural modification will be undertaken to improve the specificity and affinity for Pgp. This generic structure also offers the flexibility to prepare a wide range of molecules that are readily suitable for halogenation with either Iodine-123 or F-18 for radiopharmaceutical development. Finally these phenolic type of molecules based on Quercetin are relatively less toxic than equivalent drugs.

In this proposal an extensive research program is required to develop specific drugs for the different efflux pumps present in the body, which represent multi drug resistance. A successful outcome is critically dependent on the initial synthesis of a large number of compounds for screening. The combined effort of the three institutions will boost resources significantly to a critical level required to competitively produce successful outcomes in the project. Optimisation studies on derivatives of these flavonols will be made in parallel with the assistance of *in vitro* studies by measuring the binding of compounds to the ATP sites of Pgp. An extensive *in vitro* screening program has been established in Paris, prior to radiolabelling and *in vivo* evaluation. Structural optimisation and attachment of radionuclides to promising molecular targets will be explored using molecular modelling. Initially computational chemistry using Sybyl will be undertaken to develop a pharmacophore and to assist with the incorporation of the radionuclide in the appropriate position.

In vivo evaluation will be undertaken in specific animal models both at the University of Tours in France as well as at the Sydney Cancer Centre in Australia. PET functional imaging studies may be undertaken on successful candidates at the SHFJ in Orsay, France whilst SPECT imaging will be undertaken in both Tours and in Sydney.

In addition to intellectual property and potential commercial product(s), specific PET or SPECT radiopharmaceuticals can provide valuable information on the assessment of MDR in cancer patients through functional, non-invasive, imaging and therefore make significant contributions to the understanding of MDR. Scientific and clinical researchers from both countries identified the use of PET and SPECT functional imaging of MDR as a priority area of research. Finally the clear benefits to cancer patients include choice of treatment, with minimisation of ineffective drug treatments at an earlier stage, hence reduced drug side effects and discomfort to patients and improvements in their quality of life. There are also reduced health costs by avoiding expensive and ineffective drug treatments.

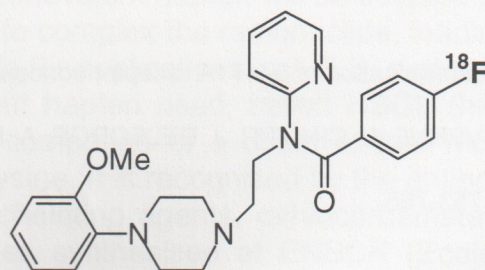
“Development of fluorine 18 labelled MPPF, radiopharmaceutical tracer for serotonergic system exploration”

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Positron Emission Tomography (PET) is a non-invasive method for exploration, in man and animals, of metabolism with radiopharmaceutical tracers labelled with positron emitters such as carbon 11 and fluorine 18 obtained with a cyclotron.

Among the ever increasing number of tracers focussed at the CNS neurotransmission, the discovery of a new family of serotonergic 5HT_{1A} antagonists (WAY 100635) has led to the first in vivo imaging of 5HT_{1A} receptors in man, located in cerebral structures such as cortex and hippocampus. Exploration of serotonin pathway is particularly interesting in normal or diseased state, as this neurotransmitter is involved in the control of mood, sleep and is probably altered in psychiatric disorders.

CERMEP, in collaboration with other PET centres has developed a new 5HT_{1A} antagonist, MPPF, labelled with fluorine 18.



MPPF, 5HT_{1A} antagonist

PET Brain tomography:
MPPF fixation in hippocampus

[¹⁸F]MPPF has the advantage of fluorine 18 labelling, with a longer half-life (110 min vs 20 min for carbon 11) and easier radiosynthesis automation. Moreover, MPPF affinity for 5HT_{1A} is close to serotonin itself, thus enabling displacement of MPPF by endogenous serotonin during pharmacological challenges.

Automated radiosynthesis of MPPF is achieved via a classical [¹⁸F]F⁻ fluoro for nitro displacement, activated by a catalyst, on a nitro precursor prepared in four steps. A final HPLC purification ensures the production of [¹⁸F]MPPF with a high purity and a high specific activity.

Ex vivo autoradiographies and PET studies in animals (rat, cat) have shown the excellent specificity of MPPF for the 5HT_{1A} receptor. Experiments with intracerebral β

probe have evidenced the displacement of [^{18}F]MPPF by endogenous serotonin after fenfluramine injection.

[^{18}F]MPPF is now used in man for non-invasive PET studies of serotonergic system. Normal volunteers matched for age and sex have been screened as a database and to compute a mathematical model of the tracer kinetic describing 5HT $_1\text{A}$ receptor affinity and availability.

Patients PET studies are underway in various cases such as epilepsy (CERMEP), psychiatric diseases, sleep disorders in particular hypersomnia/narcolepsy (Austin Hospital), where PET studies are realised with or without treatment.

Collaboration CERMEP Lyon, CRC Liège, TEP de l'hôpital Erasme Bruxelles, Austin Repatriation & Medical Centre PET unit, Melbourne. Grants from INSERM /CFR and French Embassy in Australia are gratefully acknowledged.

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J. Cereb. Blood Flow Metabol. (2002) 22(6), 753-765

"Improvement of A.E.S System, Using a ¹⁸⁸Re-Radiolabeled Hapten"

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Feasibility of two-step radioimmunotherapy (RIT) of cancer by the Affinity Enhancement System (AES) (Scheme 1.) has been demonstrated in experimental and clinical studies (1, 2, 3). This technique, associating a bispecific antibody (BsAb) and a radiolabeled bivalent peptide, reduces toxicity and improves therapeutic efficacy of the treatment compared to the one step targeting method. The formation of a cyclic complex (antigen-BsAb-hapten-BsAb-antigen) observed on the tumor allows good tumoral fixation. This is associated with low non-specific uptake, due to the fast clearance of the hapten from the organism. Iodine 131, used currently in clinical applications, has however several disadvantages. These include the mean energy of β particles, strong γ emission and long physical half-life. Rhenium-188 is the radionuclide of choice to replace iodine-131; its low cost, its availability from a W-188/Re-188 generator (4) and its very favorable radiophysical properties ($t_{1/2}=16.9\text{h}$; $E_{\beta}=2.118\text{ MeV}$; $E_{\gamma}(15\%)=155\text{keV}$) make it a very interesting radionuclide for radioimmunotherapy applications. Unlike the case of iodine-131, the radiolabelling of the peptide by rhenium-188 requires the preliminary coupling of a bifunctional chelating agent. This ensures the formation of an in vivo stable complex with the radionuclide.

The object of this post-doctoral project is to obtain a rhenium-188 radiolabeled bivalent hapten.

To do this, a monovalent hapten will be coupled to a chelating agent that involves two or three patterns to complex the radionuclide, leading to a bivalent or trivalent radiolabeled hapten, suitable for applications in A.E.S. system.

The monovalent hapten used, called HSGL (histaminosuccinylglycyllysine) is a small heteropeptide composed of a chain of four molecules which are histamine, succinic acid, glycine, lysine. It is recognised by the antibody anti-HSG (5).

Two kinds of chelating agents, dithiocarbamates and dithiobenzoates will be studied. They have been synthesised at ENSCR (Ecole Nationale Supérieure de Chimie de Rennes). For the dithiocarbamates family, the complexation of rhenium or technetium leads to a nitrido complex (6). Two molecules of dithiocarbamates are involved in the complexation (Scheme 2.). Studies of dithiobenzoates at the macroscopic scale show that three molecules of chelating agent ensure the complexation of the rhenium (7) as illustrated in scheme 3.

Two methods can be investigated to obtain this new therapeutic tool: the coupling of the hapten with the chelating agent followed by the radiolabelling, and the radiolabelling of the chelating agent followed by the coupling with the hapten.

Further, this new Re-188 hapten will be tested in vitro to check its immunoreactivity towards the antibody-679 (anti-HSG) and its stability in serum media. According to these preliminary results, biodistribution studies on rats and on the sheep model, should be then investigated.

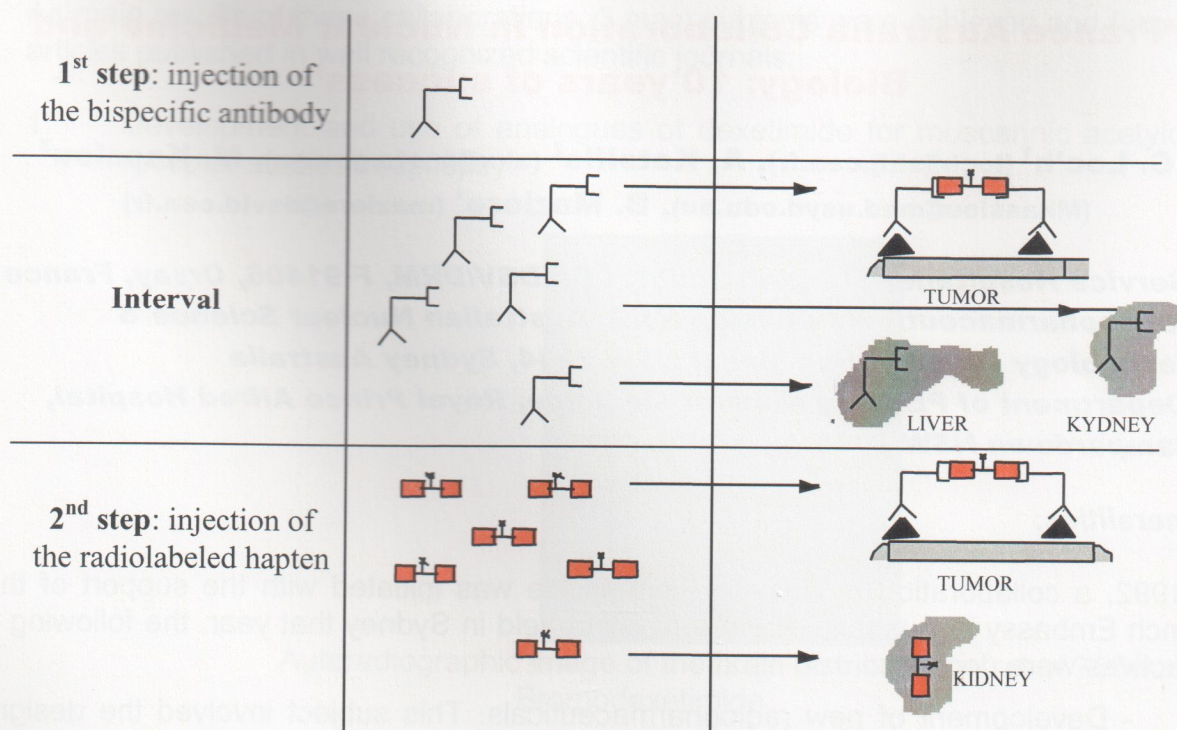
This project relies on a FAIR programm which involves a collaboration between several partners from France and Australia. The hapten has been synthesised in France, at INSERM (Institut National pour la Sante et la Recherche Medicale, Nantes) and Chelatec (Company supplying Chelating agents and vector technology, Nantes). In

Australia, the organic chemistry work (coupling the hapten and the chelating agent) occurs in Murdoch University (Perth). The radionuclides are supplied by ANSTO (Australian Nuclear Science and Technology Organisation, Sydney) and the work of radiochemistry and biodistribution studies takes place in Fremantle hospital.

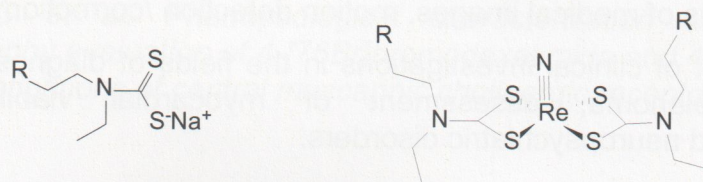
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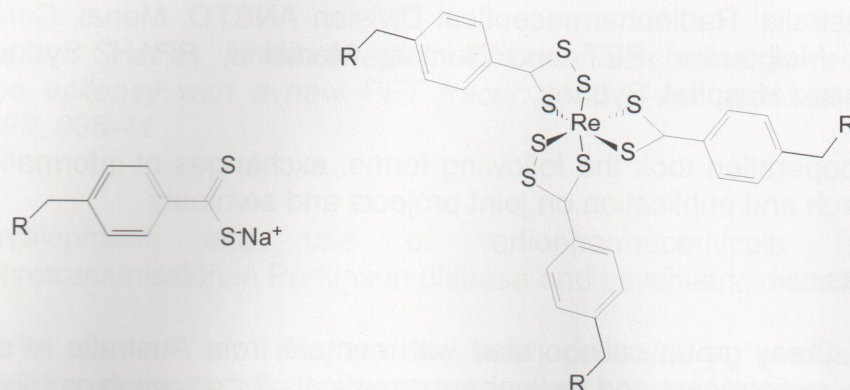
NB: Illustrations appear on next page.



Scheme 1. *Affinity Enhancement System.*



Scheme 2. *Dithiocarbamates and their rhenium complexes*
(R=coupling function)



Scheme 3. *Dithiobenzoates and their rhenium complexes.*
(R=coupling function)

“France Australia Collaboration in Nuclear Medicine and Biology: 10 years of success”

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Generalities:

In 1992, a collaboration in Nuclear Biomedicine was initiated with the support of the French Embassy in Australia. From a meeting held in Sydney that year, the following 3 objectives were decided:

- Development of new radiopharmaceuticals. This subject involved the design, synthesis and radiolabelling with positron emitters (carbon-11, fluorine-18, bromine-76) for positron tomography (PET) and with iodine-123 for single photon tomography (SPET). of novel ligands and their radiopharmacological evaluation.
- Improvement of instrumentation and data processing: measurement of input function, data analysis of medical images, motion detection /correction.
- Development of clinical investigations in the fields of diagnosis and metabolic radiotherapy of melanoma, assessment of myocardial viability, studies of neurodegenerative and neuropsychiatric disorders.

The form of the collaboration took advantage of the expertises and resources in each country. During 1992-2002 period, 11 centers from our 2 countries collaborated:

- from France :CEA-SHFJ (Orsay), INSERM U316 (Tours), CERMEP (Lyon) CEA-LETI (Grenoble), Centre J. PERRIN (Clermont-Ferrand), CHU (Nantes): CHU (Brest).
- from Australia: Radiopharmaceutical Division ANSTO, Menai, Centre for PET Austin Hospital, Melbourne, PET and Nuclear Medicine, RPAH, Sydney, Nuclear Medicine Westmead Hospital, Sydney,

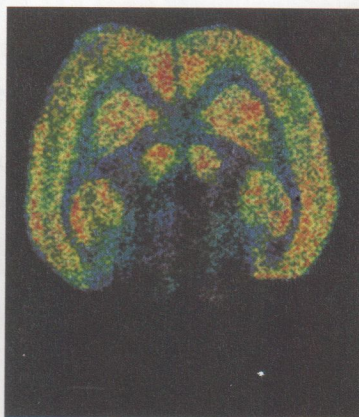
This scientific cooperation took the following forms: exchanges of information, visits of scientists, research and publication on joint projects and seminars

Scientific results:

Specifically, the Orsay group collaborated with centers from Australia to develop and characterize new radiotracers and radiopharmaceuticals for research or clinical use and to establish protocols to investigate physiological and pathological mechanisms in humans or animals, especially in the area of neurotransmission (neurodegenerative diseases, psychiatric disorders).

As main results of these collaborations, 3 main subjects were achieved and followed by articles published in well recognized scientific journals:

1. Development and use of analogues of dexetimide for muscarinic acetylcholine receptor studies in epilepsy.



Autoradiographic image of the brain distribution in rat of 76Br-Bromodexetimide

Kassiou M, et al, Synthesis of 76Br-4-bromodexetimide and 76Br-4-bromolevetimide: radiotracers for studying muscarinic cholinergic receptors using PET. *J Labelled Comp Radiopharm* 1995;3:259-266

Loc'h C et al, Pharmacological characterization and positron emission tomography evaluation of 4-[76Br]bromodexetimide and 4-[76Br]bromolevetimide for investigations of central muscarinic cholinergic receptors. *Nucl Med Biol.* 1996 235-43.

Kassiou M et al, Radiosynthesis of [123I]N-methyl-4-iododexetimide and [123I]N-methyl-4-iodolevetimide: in vitro and in vivo characterisation of binding to muscarinic receptors in the rat heart. *Nucl Med Biol.* 1996 :147-53.

Strijckmans V et al, In vitro pharmacological properties of 4-bromodexetimide for muscarinic receptors. *Life Sci.* 1996;PL337-44.

Dupont S. et al, In vivo imaging of muscarinic cholinergic receptors in temporal lobe epilepsy with a new PET tracer: [76Br]4-bromodexetimide. *J Nucl Med.* 1999, 935-41

2. Development and use of radiopharmaceuticals for dopaminergic neurotransmission in Parkinson disease and psychiatric disorders.

Loc'h C. et al, Preparation and preliminary evaluation of 3-threo-[76Br]bromomethylphenidate enantiomers: potential tracers for PET study of dopamine transporters, *J Label Compound Radiopharm*, 1999, S42-444

Kassiou M et al, *Radiosynthesis of bromide-76 and iodine-123 labelled enantiomers of A-69024 : radioligands for dopamine D1 receptors studies using PET and SPECT. J Label Compound Radiopharm, 2000, 43 :339-346*

Kassiou M et al, *(+)-[76Br]A-69024: a non benzazepine radioligand for studies of dopamine D1 receptors using PET, Nucl Med Biol 2002, 295-302*

3. Development and use of analogues of epibatidine for nicotinic acetylcholine receptor studies in Alzheimer diseases.

Kassiou M et al, *Preparation of a bromine-76 analogue of epibatidine, J Labelled Comp Radiopharm, 2002, in press*

Kassiou M et al, *Pharmacological evaluation of a Br-76 analogue of epibatidine: a potent ligand for studying brain nicotinic acetylcholine receptors, Synapse, 2002, 45, 95-104*

Future collaborations:

In the basis of our common experience, a number of areas of high interest were identified during the meeting held in Sydney in April 2002:

- Study of the nicotinic acetylcholine neurotransmission using a new radiopharmaceutical : F-18 A 35380 in tobacco addiction and Alzheimer disease.
- Measurement of endogenous dopamine with C-11 A-69024,
- Development of NMDA ligands labelled with C-11 and F-18 for clinical studies in Alzheimer's disease, Parkinson's disease, trauma, stroke, epilepsy
- Follow up of graft therapy in neurodegenerative diseases by measurement of peripheral benzodiazepine receptors with a new class of radiopharmaceuticals.

SESSION 3

“Value of sestamibi for the prediction of resistance to primary chemotherapy”

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Neoadjuvant chemotherapy can cause significant tumor regression of primary breast carcinoma, thus allowing a more conservative surgery to be performed in bulky tumors, even if a benefit in terms of prolongation of survival remains to be established. Clinical resistance is often encountered, but is not apparent during the first months of treatment. This has stimulated the search for tests predictive of tumor response in order to more accurately select patients who may benefit from such a long, risky in terms of side effects, and costly therapy.

Several mechanisms have been reported to be involved in the multidrug resistance (MDR) to many of the currently used chemotherapeutic drugs and specific proteins have been demonstrated to be overexpressed in these tumor cells. The most important seems to be a membrane transporter, P-glycoprotein (Pgp), product of the *MDR1* gene in man. Like the other members of that family, it can actively extrude a wide variety of substrates from the cells, among which anthracyclines as well as other small lipophilic cations. Drugs called reversers are able to block the efficacy of these transmembrane resistance mechanisms. In patients with a tumor resistant to chemotherapy, such a reverser could make the tumor sensitive to chemotherapy. However, up to now, most of the reversers have not yet demonstrated their clinical efficacy, often because of their side effects. Since resistance to chemotherapy as well as the possible efficacy of a reverser are processes that take months before becoming evident either clinically or by conventional imaging, any technique that could detect these effects during the early phase of the treatment or, even better, that could *predict* the outcome would save lives, time and money.

The radiopharmaceutical hexakis (2-methoxyisobutylisonitrile) technetium (^{99m}Tc -sestamibi), which has been initially registered for myocardial blood flow imaging, then for breast cancer imaging, is an organometallic, small lipophilic cation which had been shown to be a multidrug resistance Pgp transport substrate and can be utilized as a functional probe of Pgp transport activity in solid tumors (1). We have measured (2) the 2-hr retention of ^{99m}Tc -sestamibi and ^3H -daunomycin in MDR-sensitive and MDR-resistant cultured tumor cells (KB-3-1 and KB-A1, respectively). Retention of both radioactive molecules was decreased of $64 \pm 11\%$ and $69 \pm 5\%$ (N.S.), respectively, suggesting that the modification of intracellular level of ^{99m}Tc -sestamibi induced by the overexpression of MDR closely reflects that of ^3H -daunomycin. This confirms that ^{99m}Tc -sestamibi could be used as a surrogate to assess the tumor retention of anthracyclines. In another in vitro study (3), we have compared the cellular retention of the same two agents in the same cell lines now incubated with one of three recognized MDR-reversing agents, namely, verapamil, PSC833 and S9788. While a reversing effect on the retention of ^3H -daunomycin was observed with each reversing agent, there was either no effect or only a faint increase on the retention of ^{99m}Tc -sestamibi. These results suggest that ^{99m}Tc -sestamibi *cannot* be used as a faithful surrogate to assess

the effect of MDR-reversing agents on the tumor retention of anthracyclines. At variance with these results, Tatsumi et al. (4) have recently reported that, in mice resistant grafted tumors, ^{99m}Tc -sestamibi washout rate could be totally reversed by a different reversing agent, MS-209. In humans, data mostly originate from Del Vecchio et al. (5) who demonstrated that 4-hr residual tumor retention of ^{99m}Tc -sestamibi was 56% in sensitive breast cancers vs. 25% in MDR-resistant tumors. Further studies have focused on the possible role of ^{99m}Tc -sestamibi scintigraphy in the *early detection* of resistance during the first cycles of primary chemotherapy. In breast cancer, Maini et al. (6) have reported a 100% specificity and Mankoff et al. a 100% specificity (7). For Tiling et al. (8), "sestamibi is as useful as FDG-PET for the monitoring of tumour response to neoadjuvant chemotherapy". Another potential application of the technique would be to *predict* chemoresistance *before* the first cycle of neoadjuvant chemotherapy. In malignant lymphomas, Dimitrakopoulou-Strauss et al. have compared sestamibi and FDG (9). They concluded that "while PET studies with FDG prior and after chemotherapy are required to determine the effect of a cytostatic treatment, one study with sestamibi prior to therapy may be adequate for estimating the result of therapy". In our own reported experience (10) of ^{99m}Tc -sestamibi scintimammography performed before any treatment in a series of 45 patients with a T2 > 3 cm breast cancer, specificity was also 100% when compared with pathology. This confirms the high potential of ^{99m}Tc -sestamibi scintimammography. We are currently validating an automatic measurement of the tumor-to-background ratio in order to provide any user with a reproducible quantitative criteria for an objective assessment of the predictive response.

The use of ^{99m}Tc -sestamibi in breast cancer opens the way to the truly functional and predictive evaluation of tumor response to chemotherapy by a non invasive imaging technique, an important issue in an era where the cost of chemotherapy is increasingly high and where unnecessary treatments should be avoided as much as possible for ethical as well as economic reasons.

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"Clinical PET: Changing the Practice of Oncology"

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ABSTRACT

Until recently positron emission tomography (PET) has been largely confined to academic institutions with the capital and human resources to support this technologically advanced modality. Despite the great promise shown by this technique for applications in neurology and cardiology, more recently its utility in oncology has fuelled the wider dissemination of this modality into routine clinical practice. Although initially stifled by lack of availability and the high cost of clinical investigations, recently reimbursement has become available in most countries on the basis of compelling evidence of the clinical efficacy of PET in evaluation of a broad range of cancers. In addition to the widespread use of the glucose analogue, fluorine-18 fluorodeoxyglucose (FDG), new positron-emitting tracers now offer exciting opportunities to better characterise tumour biology *in vivo*. Providing a link between the revolution in molecular biology and the clinical practice of oncology, PET tracers can now be applied to translational research. Small animal PET scanners allow tracers to be validated prior to use in human subjects. The Peter MacCallum Cancer Institute clinical PET program began operation in 1996 and since that time has grown to be the first Australian centre with 2 PET scanners, including the first combined PET/CT. Although the majority of the almost 10,000 studies performed in our facility have utilised FDG, new tracers are increasingly being used in clinical trials, particularly for therapeutic monitoring of novel chemotherapeutic agents.

INTRODUCTION

Positron emission tomography (PET) provides physiological and metabolic characterisation of tissue and organ function by imaging the *in vivo* distribution of chemicals or pharmaceutical agents, labelled with positron emitting radionuclides. The physical characteristics of positron decay allow high sensitivity and high resolution imaging (1). This, coupled with the physical characteristics of the positron emitting radionuclides which allow production of a diverse range of biologically-relevant radiopharmaceuticals (2), makes PET a powerful investigational modality for a diverse range of diseases.

The first human PET scanner was installed at the University of Washington in St. Louis, Missouri in the mid 1970's. In the ensuing fifteen years PET was almost exclusively a research tool confined to large academic institutions capable of supporting the large capital and technical support requirements of this modality. The early PET scanners provided only a small number of slices, practically limiting studies to a single region or organ. Nevertheless, during this period, data were acquired which suggested the possible clinical utility of this modality.

Initial PET studies evaluated F-18 fluorodeoxyglucose (FDG) extending on the deoxyglucose methodology developed by Louis Sokoloff for autoradiographic studies of

cerebral metabolism (3). Pioneering studies performed by a number of groups evaluated a range of neurologic conditions including epilepsy, stroke and dementia. These studies suggested that PET provides unique metabolic characterisation of these disease processes by demonstrating regional, cortical hypometabolism in relationship to these conditions. Investigation of cerebral tumours demonstrated increased metabolism in relationship to gliomas with increasing intensity of activity noted in high grade tumours providing a non-invasive means of grading tumours (4) and allowing differentiation of recurrent tumour from radiation necrosis (5). These studies paved the way for further evaluation of PET scanning for oncologic applications.

PET scanning was also extensively evaluated for cardiac applications, particularly including ischaemic heart disease (6). The demonstration of enhanced FDG retention in areas with severe impairment of myocardial perfusion (perfusion-metabolism "mismatch") was demonstrated to be predictive of recovery in cardiac function following revascularization and has become widely accepted as the gold standard for evaluation of myocardial viability in areas with contractile dysfunction. By the early 1990's, it was apparent that PET had a potential clinical role in the localisation of epileptic foci in patients with temporal lobe epilepsy (7) and in assessing myocardial viability (8).

Over the ensuing 10 years, there has been increasing recognition of the potential role of FDG PET scanning in oncology. It has been shown that there is increased uptake and retention of this tracer in a diverse range of tumours. Technical advances in PET scanners including development of algorithms for whole body imaging (9) using a PET scanner have made clinical PET studies for oncology patients more practical. The emerging oncological applications of PET scanning have been reflected in an increasing proportion of clinical PET studies at most centres world wide being performed on the patients with known or suspected cancer.

In late 1996, the Peter MacCallum Cancer Institute (PMCI) installed its first PET scanner followed by a second scanner in early 2001. Both these scanners were based on sodium iodide detectors (10) which are less expensive to manufacture and require lower amounts of FDG to be administered to patients (11). In late 2001, the initial scanner was replaced by the first combined PET/CT scanner to be installed in Asia. Almost 10,000 patient studies have been performed in our facility including over 1,500 on the combined scanner.

THE ROLE OF FDG PET IN ONCOLOGY

The major factor in the growth of oncologic PET has been the high uptake and retention of the glucose analog, FDG in a wide range of malignancies leading to high sensitivity for the detection of disease and, as a corollary of this, a high negative predictive value. Although FDG is also concentrated in inflammatory tissue, the specificity and positive predictive value of this agent has also been shown to be high in most oncological settings (12).

However, in view of the relatively high cost of PET studies, appropriate use of PET scanning requires definition of those situations where PET provides incremental diagnostic information compared with standard diagnostic algorithms. Like all diagnostic tests, the use of Bayesian principles should be used to identify individuals most likely to benefit from the potentially superior diagnostic capabilities of PET compared with other

modalities. Take, for example malignant melanoma. A patient with thin primary lesion (stage 1) on the lower limb has a very low likelihood of having or ever developing, metastatic disease. PET scanning, CT, MRI, ultrasound, sentinel lymph node biopsy and clinical examination are all likely to be negative but clearly, the last option is the cheapest. Another patient with a thick melanoma on the trunk and palpable lymph node in the axilla (stage 3) has a intermediate to high likelihood of having or developing systemic metastatic disease, even in the absence of clinical symptoms or signs. In such patients, exclusion of systemic metastases by a whole body screening technique would be appropriate. The more sensitive and specific that technique is, the greater the likelihood of impacting management. Somewhere between these two scenarios, the choosing the correct balance between cheap clinical surveillance, aggressive locoregional staging and comprehensive systemic staging becomes more difficult.

In establishing our facility we have sought to influence referral patterns to those situations where epidemiological and case control data suggest that conventional diagnostic algorithms currently fail us. This is particularly the case in situation where recognition of this failure leads to routine use of either a morbid procedure or treatment in an entire population of patients, even in the absence of abnormality after conventional staging. For example, CT scanning is recognised to have insufficient accuracy for staging the status of mediastinal lymph node spread of non-small cell lung cancer to determine operability. Accordingly, a large number of patients undergo mediastinoscopy and pathological sampling of lymph nodes. Other patients are subjected to futile open and close thoracotomies due to incorrect staging. FDG PET has convincingly been shown to be more accurate than CT for staging the mediastinum (13) and in a recent randomised control trial was shown to significantly reduce unnecessary thoractomies (14).

By trying to limit the use of PET to situations where a range of different management options are available depending on the true extent of disease, we have been able to demonstrate that PET has a high and appropriate impact on management. Our group have recently published data on the clinical impact of PET in specific diseases including the staging of NSCLC (15), the restaging of NSCLC (16), the staging of cervical cancer (17), the restaging of colorectal cancer (18), the diagnosis of solitary pulmonary nodules (19), evaluation of lymphoma (20) and malignant melanoma (21).

LIMITATIONS OF FDG PET AND NEW TRACERS TO ADDRESS THESE

The uptake of FDG in inflammatory conditions, leading to false positive results, is regarded as one of the major limitations of this tracer in oncological settings (22). In an attempt to address this limitation agents that look at biological processes more specific to cancers than is glycolytic metabolism, have been evaluated. These include amino acid analogues that trace protein synthesis (23), thymidine analogues that evaluate cellular proliferation (24), and markers of cellular sterol metabolism (25). An additional feature of cancers is the presence of hypoxia. PET tracers such as F-18 fluoromisonidazole have been shown to be taken up in a high percentage of some tumours that clinical and experimental evidence suggests are likely to be hypoxic and because of this, more resistant to conventional therapies (26).

Additional potential limitation of FDG is the potential for an increase in uptake during the process of apoptosis, an energy requiring process, that may limit the utility of early scanning to determine therapeutic response. The so-called "metabolic flare" has been

described (27) as actually being predictive of therapeutic response in breast cancer patients treated by hormone manipulation.

Due to issues related to synthesis times and providing adequate tracer amounts for several studies at least, we have focussed on fluorinated PET tracers rather than shorter-lived tracers using carbon-11. Currently the Peter MacCallum Cancer Institute Centre for Molecular Imaging has active clinical research trials using the cellular proliferative tracer, F-18 fluorothymidine (FLT), and the hypoxia markers F-18 fluoromisonidazole and F-18 fluoroazamycin arabinoside (FAZA). We also have preclinical experience with F-18 fluoroethyltyrosine (FET). Our radiochemistry program is developing the facility to provide F-18 fluorocholine (FCH) and in the longer term has significant interest in I-124 and Cu-67 tracers.

FUTURE DIRECTIONS

The advances in molecular biology and availability of DNA micro-array are increasing our understanding of cancer rapidly and identifying new cancer targets for both imaging and therapy. Nuclear medicine in general and PET in specific, are uniquely placed to capitalise on this knowledge by applying established tracer kinetic approaches to disease evaluation in vivo. As more and more basic oncological research is being performed in animal models, particularly including immunocompromised and transgenic mice, it is logical to apply functional imaging techniques also to this research (28). We believe that such translational research will be an important adjunct to clinical PET particularly in the area of drug evaluation. Towards this end, the Peter MacCallum Cancer Institute is installing the first small animal PET scanner in Australia, later this year.

CONCLUSION

Molecular imaging is revolutionising the diagnosis, staging, surveillance and therapeutic monitoring of cancer. Increasingly, its ability to characterise the basic biological features of cancer will provide vital in vivo bioassays that will both aid prognostic stratification and guide selection of the most appropriate treatment. Such treatment selection will be made feasible at an individual patient and possibly individual lesion level rather than our current approach of treating all patients on the basis of broad disease groupings.

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“Radioimmunotherapy with Iodine-131 Anti-CD20 Chimeric Monoclonal Antibody (Rituximab) for relapsed or refractory Non-Hodgkins Lymphoma”

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Radioimmunotherapy of relapsed or refractory low grade non-Hodgkins lymphoma (NHL) with anti-CD20 murine monoclonal antibodies such as ¹³¹I-tositumomab (Bexxar®) or ⁹⁰Y-ibritumomab (Zevalin®) has demonstrated objective response rates (ORR) of 75 – 80% and complete remission (CR) in 15 – 40% of patients. These responses are significantly better than anti-CD20 immunotherapy with rituximab (Rituxan®) with ORR 45% and CR 5% in comparable NHL patients. However, grade IV toxicity has been observed, thrombocytopenia 4% and 9%, and neutropenia 20% and 30%, of patients treated with Bexxar® and Zevalin® respectively. These murine monoclonals are also immunogenic, and HAMA formation in 10 – 20% of patients after a single treatment of refractory NHL may preclude subsequent radioimmunotherapy on relapse. We have radioiodinated chimeric IgG₁ anti CD20 Mab in an ongoing physician-sponsored Phase II Clinical Trial of ¹³¹I-rituximab radioimmunotherapy of relapsed/refractory low grade NHL in 32 patients followed up 6 months to 2 years. The ORR is 78% and clinical CR 50%, duration yet to be determined. Tracer dosimetry studies in each patient limit the whole body radiation absorbed dose to 0.75 Gy. There has been no instance of grade IV haematological toxicity and mild/moderate myelosuppression was self-limited. Repeat treatments in 3 patients showed similar responses without significant myelotoxicity. An Australia-wide multicentre phase II clinical trial is showing comparable preliminary results in the small numbers of patients currently evaluable.

"Iodobenzamides, agents with an affinity for melanoma: applications to diagnosis, staging and therapy"

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Melanoma is a neoplasia of dramatically increasing incidence that has propensity to spread rapidly. Early detection is fundamental and patient management requires reliable, sensitive and reproducible staging methods, such as a single examination by planar scintigraphy or single-photon emission tomography (SPET) using a radiopharmaceutical with a selectivity for melanoma tissue. We have been the first to propose a series of iodobenzamide derivatives showing an affinity for melanoma tissue. A first compound, [¹²³I]-N-(2-diethylaminoethyl)-4-iodobenzamide (BZA), selected after preclinical studies in melanoma-bearing mice showed promising results in a trial as an imaging agent of primary melanomas and metastases in 159 patients. Only the time with the best tumoral image definition was to be improved: 18-24 h after administration. From these results, multiple procedures were developed: i) Pharmacochemical studies in order to select a derivative with a better pharmacokinetic profile. ii) Determination of the uptake mechanisms of iodobenzamides in melanoma tissue. These different procedures and main results will be presented. Some of the BZA analogues exhibited an original experimental behaviour and an affinity for melanic pigment was evidenced.

Finally, a new compound, N-(2-diethylaminoethyl)-2-iodobenzamide (BZA₂) was selected for a phase II clinical trial in view of its pharmacokinetic experimental profile in melanoma-bearing mice. We will report the results of a trial of ¹²³I-BZA₂ as a melanoma imaging agent using planar whole-body scintigraphy in forty patients. Performance was evaluated in patients classified with primary ocular lesions (n=12), metastases of ocular or cutaneous origin (n=15) or with no known secondary lesions (n=13), and results were compared with conventional investigation techniques. No clinical or biological adverse events were recorded and good quality images were obtained four hours after administration. After a follow-up of more than one year, the overall results on a per patient basis showed a sensitivity of 79%, a specificity of 95%, a positive predictive value of 94% and a negative predictive value of 83%. The four false negatives observed were ocular lesions (three with a thickness ≤2.9 mm and one achromic). Interestingly, all the proven secondary lesions were imaged with a sensitivity of 100%, a specificity of 89% and a negative predictive value of 100%. In eight patients, the absence of tracer fixation was confirmed as truly negative, and for one patient with no suspected metastasis, a gastric image was evidenced. Hence nine diagnoses would have been modified by ¹²³I-BZA₂ scintigraphy data. This tracer allowed a discrimination between benign and malignant lesions and in malignancies between those of melanoma origin and others. Selective for melanoma tissue, it appeared promising for differential diagnosis, staging and restaging of melanoma. Moreover, properties of some of the analogues exhibit a potentiality for the targeting of melanoma tissue giving them an interest in therapy of this pathology.

"SENTINEL LYMPH NODE BIOPSY: Clinical Relevance"

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Sentinel lymph node biopsy (SLNB) has become an important technique in the management of patients with intermediate level melanoma, clinical operable breast cancer and some other cancers. The technique relies on lymphatic mapping to define the lymph drainage from a primary tumour with the premise that the lymph nodes, which directly drain from that area, will reflect the tumour status of the remainder of the node field. Current techniques use lymphoscintigraphy where a radioactive labelled particle and / or blue dye are injected intradermally or intraparenchymally to map the lymph drainage, often in conjunction with a radioactive gamma probe at surgery.

Lymphoscintigraphy:

Melanoma:

In patients with melanoma the SLNB has improved the staging and prognostic information by more accurate determination of whether regional lymph nodes have metastatic spread. This has a major impact on patient management as those patients with negative nodes do not require regional lymph node dissection and have a significantly better prognosis. In our experience of over 3000 patients the combined sentinel node biopsy technique localised accurately 98% of sentinel lymph nodes.

Technique:

Lymphoscintigraphy in patients with melanoma to locate the sentinel lymph nodes involves the intradermal injection of a radiocolloid around the melanoma site or the excision biopsy site. Injections of 5 –10 MBq in 0.05-0.1ml/inj are used and typically 4 injections are usually required. Following tracer injection dynamic imaging is performed to follow the lymphatic collecting vessels until they reach the draining sentinel nodes. An image should be acquired as the vessels reach the node field so that the sentinel nodes directly receiving the channels can be identified and distinguished from any second tier nodes which may sometimes be seen. Delayed scans are performed 2 hours later at which time all regions which can possible drain the primary melanoma site are examined with 5-10 minute static images. The surface location of all sentinel nodes is marked on the skin with indelible ink and a permanent point tattoo of carbon black ink. The depth of the sentinel node from the skin mark is measured in an orthogonal view with a radioactive marker placed on the skin mark. At surgery blue dye is used as a mapping technique and the sentinel nodes are also checked by using a gamma probe to confirm the true sentinel nodes and that the sentinel nodes have been removed.

Breast Carcinoma:

Several lymphoscintigraphic techniques have been described where the tracer is injected peritumorally, intratumorally or intradermally. We favour the peritumour injection technique under ultrasound control. The lymphatic drainage in breast carcinoma is predominantly to axillary lymph nodes but also to internal mammary lymph nodes (IM) and occasionally to intramammary, infraclavicular and supraclavicular node fields. To most accurately define the lymph drainage all sentinel nodes should be found. In our

experience the axillary sentinel nodes were accurately staged in 96% of patients. There is considerable variation in detection of internal mammary nodes with the highest detection being found when using small particles and delayed imaging. There is controversy over the significance on the change of management regarding internal mammary lymph involvement. In our experience the axilla drains 93%, internal mammary 45%, supraclavicular nodes 13% and intramammary nodes 12%.

Technique:

Four peritumoral injections of Tc99m antimony sulphide colloid are injected under ultrasound control. The patient performs breast massage for 15 minutes. Initial scanning is performed followed by delayed scanning at 2 hours. Delayed imaging is important, as IM nodes may not show until later in the study. The sentinel nodes are marked and tattooed as in the melanoma technique. At surgery similar techniques are used with blue dye and the gamma probe to confirm the sentinel nodes and their removal.

Surgery:

Optimal sentinel lymph node biopsy uses all the mapping techniques of lymphoscintigraphy, blue dye technique and gamma probe at surgery. The blue dye and gamma probe techniques also require considerable expertise by the surgeon. After the SLN have been removed they are subjected to histopathology and immunohistochemistry. If the SLN is positive then a therapeutic lymph node dissection is recommended. Using the SLNB in this way means that approximately 70-80% of patients with intermediate melanoma will not require full regional node dissection with its inherent morbidity. Similar results in clinical node negative breast cancer show encouraging results again potentially saving approximately 70% of patients from having an axillary lymph node dissection with its possible complications.

Histopathology and Immunohistochemistry:

The sentinel nodes are removed at surgery and sent for pathology. As there are a limited number usually 1-3 sentinel nodes this allows for more specific histopathology and immunohistochemistry. Focused histopathology allows more specific and accurate detection of micrometastases.

Other Cancers:

SLNB has also been used in vulval, penile, head and neck, thyroid, oropharyngeal cancers and also intraoperatively in colon cancer.

Comment:

The techniques require a sophisticated integration of a multidisciplinary team with significant learning curves for the surgeons, nuclear medicine physicians, technologists and pathologists. There has to be good communication between all groups with precise imaging and surgical techniques. With lymphoscintigraphy there are many techniques described and the literature reports excellent results in major centres. With lymphoscintigraphy in Australia we use small particle colloids (Tc99m antimony sulphide colloid) which allows excellent definition of lymph channels and more accurate determination of sentinel nodes in all areas. There is a significant number of unusual and unexpected drainage patterns found in melanoma many of which would be undetectable if only using a gamma probe alone or delayed imaging with large particles. There is also some controversy in breast cancer on the most accurate sentinel node technique and the relevance of extra axillary drainage sites eg internal mammary chain.

The sentinel lymph node mapping with lymphoscintigraphy and blue dye in association with applications of the gamma probe at surgery for accurate localisation of sentinel nodes is making a significant impact on patient management. This is in particular by more accurate staging and prognostic information with better stratification of patients requiring further regional node dissection and those suitable for adjuvant therapy and specific clinical trials.

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“Pharmaceutical production of nano particles using supercritical or dense gas technology”

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Introduction

The primary aim of our proposed research is to develop pharmaceutical formulations with enhanced pharmacokinetics and increased bioavailability. The particular drug delivery systems of interest are, oral, aerosols, injectable and topical with well-recognised and distinct problems of bioavailability.

More than 40% of all drugs in the USP or BP are insoluble or have some problem with solubility. It is estimated in 2000, the total combined sales of drugs that are insoluble or poorly soluble was US\$37 billion.

Precise and predictable drug delivery is made more possible by producing uniform micron size particles or powders, which can improve the efficiency and effectiveness of therapeutical formulations.

Hence the purpose of micronisation is to increase bioavailability and also to allow other modes of administration, eg insulin is a protein, which is an injectable for the treatment of diabetes, but recently particles of 1-4 microns of insulin are in phase 3 clinical trials to deliver the drug to diabetics as an inhalant.

In addition aerosolised drugs such as mucolytics, antibiotics, antiinflammatory drugs and hormones have recently been trailed. Finely powdered pharmaceuticals are however difficult to process by current techniques. In spray drying the operating temperatures are often too high for heat sensitive drugs. Thermal degradation of compounds can also be experienced in milling due to high rates of shear and requires high energy inputs and do not produce particles within a narrow range distribution.

A relatively new technique which has been used and developed by Eiffel Technologies to produce uniform micron and sub micron size particles is a dense gas process in which the gas is used as an antisolvent to precipitate compounds from solution.

Pharmaceutical processing with dense gas is relatively new and is an efficient process for producing high purity micronised particles with defined morphological structures and with a narrow size distribution rate.

Dense gas technology using fluids, near or above the critical point, as a solvent or antisolvent have been developed in recent years.

Eiffel has considered various dense gas methods as in the production of nano particles.

The first method is known as **Rapid Expansion of Supercritical Solutions (RESS)**, and involves expanding a supercritical solution of the drug through a nozzle. Whilst providing very effective methods of producing fine particles, the application of the RESS method is limited by the low solubility of drugs in dense carbon dioxide (which is usually the gas of choice since it is operated at moderate critical temperature of 31.1 degrees centigrade).

The second method, known as **Gas Antisolvent Process (GAS)**, involves rapid precipitation of the drug from organic solutions, typically using carbon dioxide as the antisolvent.

The third mode which is called the **Aerosol Solvent Extraction System (ASES)**, involves continuous introduction of a solution containing the drug of interest through a nozzle into a flowing dense gas stream (figure 1 shows the various supercritical techniques and nano particles produced by ASES) The fourth mode is **precipitation** of the drug from **gas saturated solution** called (PGSS).

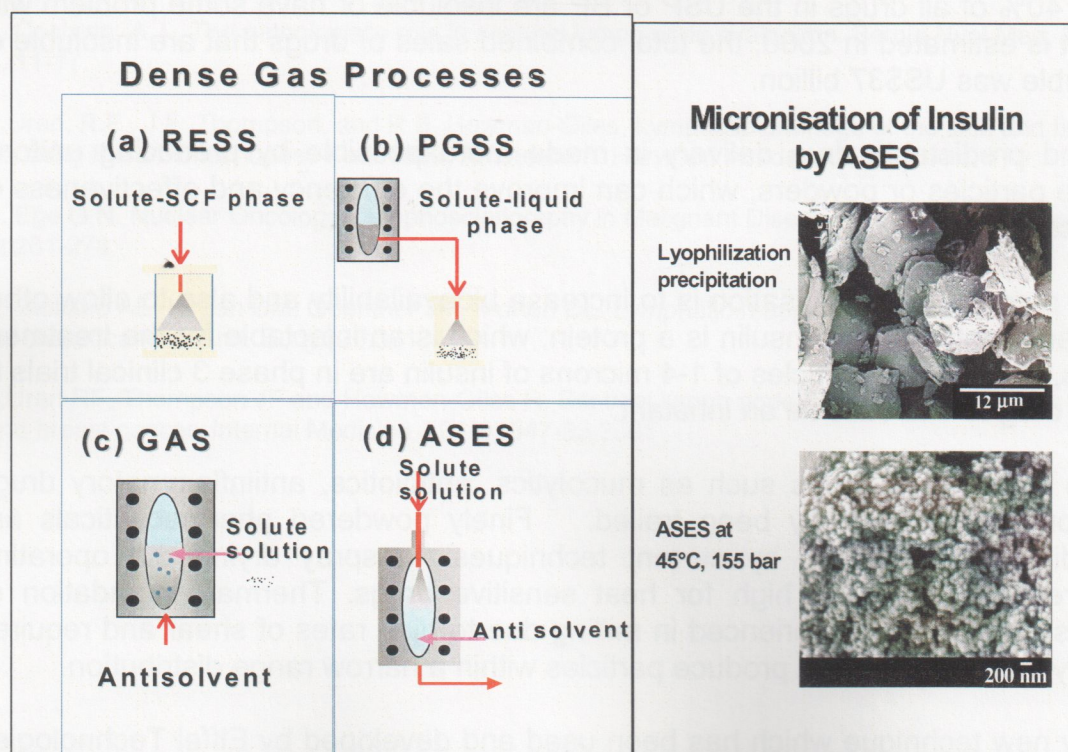


Figure 1

Figure 2 shows two different methods of producing nano particles of an insoluble nonsteroidal antiinflammatory drug and it indicates that different methods produces different morphology when comparing GAS and ASES and by producing nano particles it increases the dissolution profile of the drug and hence increases its solubility

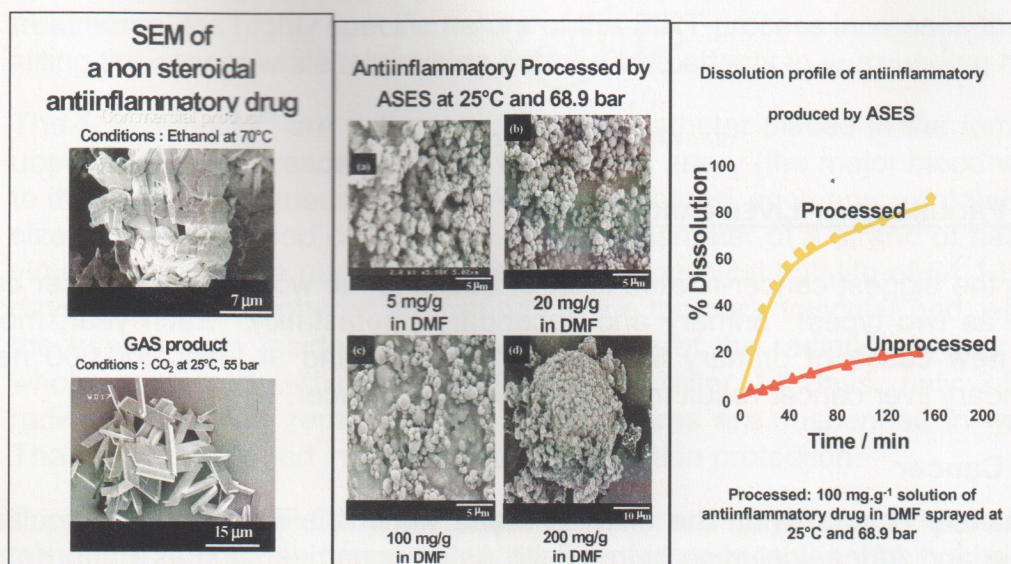


Figure 2

Figure 3 shows an immunosuppressive drug, which was processed by RESS and PGSS and shows the respiratory fraction produced by either method.

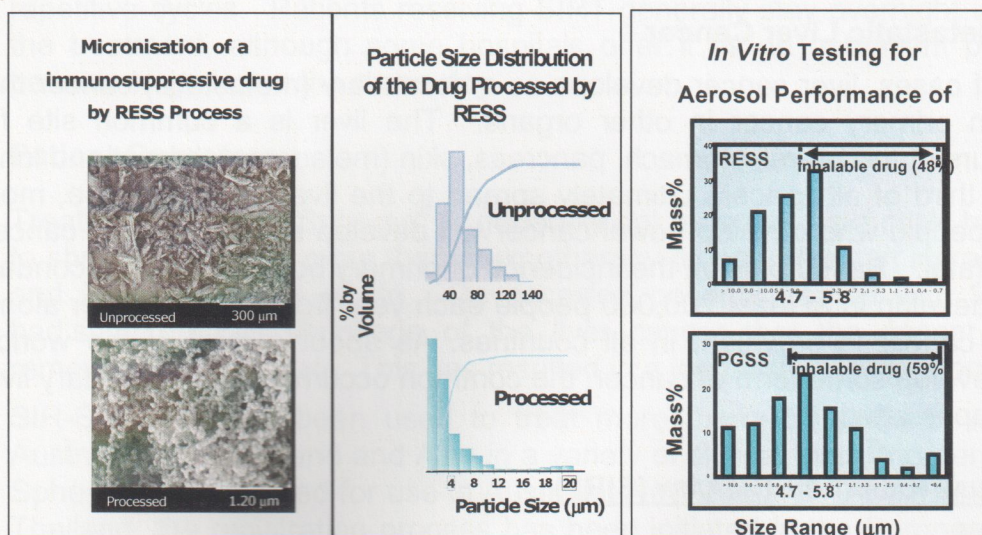


Figure 3

“Liver Cancer & Selective Internal Radiation Therapy”

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INCIDENCE AND PROGNOSIS OF LIVER CANCER

Liver cancer is the biggest cancer-related killer of adults in the world. Liver cancer can be considered as two types: primary and secondary (metastatic). Each year, more than 400,000 new cases of primary liver cancer develop and at least 200,000 new cases of secondary liver cancer result from primary bowel cancer.

Primary Liver Cancer

Primary liver cancer occurs when the disease starts within the liver. It is particularly common in Asia and Africa, including high wealth Asian countries such as Hong Kong, Japan and Taiwan. In some countries, primary liver cancer is more common than any other form of cancer and is driven largely by the very high incidence of hepatitis endemic in those countries. More than 400,000 individuals worldwide are affected by primary liver cancer each year.

Secondary or Metastatic Liver Cancer

In the majority of cases, liver cancer develops as a secondary (metastatic) cancer that has spread from primary cancer in other organs. The liver is a common site for secondary tumours of the bowel, stomach, pancreas, skin (melanoma), breast and lung; approximately a third of all cancers ultimately spread to the liver. For instance, more than 40% of all people who develop bowel cancer will develop secondary liver cancer, which will prove fatal. Based only on the incidence of primary bowel cancer, secondary liver cancer will develop in at least 200,000 people each year from bowel cancer alone. Secondary liver cancer is prevalent in all countries. As about a third of the world's population will develop some form of cancer, the common occurrence of secondary liver cancer can be appreciated.

SELECTIVE INTERNAL RADIATION THERAPY (SIRT)

Selective Internal Radiation Therapy (SIRT) is a revolutionary treatment for advanced liver cancer that utilises new technologies designed to deliver radiation directly to the site of tumours.

Conventional radiotherapy can only be applied to limited areas of the body and adversely affects nearby tissues. SIRT, on the other hand, involves the delivery of millions of microscopic radioactive spheres called SIR-Spheres[®] directly to the site of the liver tumour/s, where they selectively irradiate the tumours. The anti-cancer effect is

concentrated in the liver and there is little effect on cancer at other sites such as the lungs or bones.

SIRT Process

The SIRT process enables the specific targeting and destruction of tumours within the liver that are otherwise inaccessible. Unlike current, conventional chemotherapy treatments, the highly specific nature of the SIRT process increases the effectiveness in killing the cancer while minimising harmful side effects to surrounding healthy tissue.

The SIR-Spheres[®] are delivered through a catheter placed in the femoral artery of the upper thigh and threaded through the hepatic artery (the major blood vessel of the liver) to the site of the tumour. The microscopic spheres, each approximately 35 microns (the size of four red blood cells or one-third the diameter of a strand of hair), are bonded to yttrium-90 (Y-90), a pure beta emitter with a physical half-life of 64.1 hours (about 2.67 days). The microspheres are trapped in the tumour's vascular bed, where they destroy the tumour from inside. The average range of the radiation is only 2.5 mm, so it is wholly contained within the patient's body; after 14 days, only 2.5 percent of the radioactive activity remains. The microspheres are suspended in water for injection. The vials are shipped in lead shields for radiation protection.

Treatment with SIR-Spheres[®] exploits a normal physiological process to selectively target the cancerous tissue. Healthy liver tissue receives most of its blood supply via the portal vein, while liver tumours receive the overwhelming majority of their blood supply from the hepatic artery. The consequence of this is that catheterisation of the hepatic artery permits the targeting of therapeutic material to liver cancer.

SIRT is a single targeted procedure, unlike chemotherapy, which is administered in repetitive cycles. Patients receiving SIRT generally stay overnight in hospital following the treatment, although some hospitals offer it as an outpatient procedure, enabling patients to go home the same day.

Patient Outcomes

Treatment with SIR-Spheres[®] is generally not regarded as a cure, but has been shown to shrink the cancer more than chemotherapy alone. This can increase life expectancy and improve quality of life. On occasion, patients treated with SIR-Spheres[®] have had such marked shrinkage of the liver cancer that the cancer can be surgically removed at a later date. This has resulted in a long-term cure for some patients.

SIR-Spheres[®] has been used to treat more than 750 patients with liver cancer in Australia, New Zealand and Asia in a variety of clinical trials and general practice. SIR-Spheres[®] is registered for use in Australia, New Zealand, USA, Hong Kong, Singapore, Thailand; the registration process has been initiated in the European Union, Malaysia, and Taiwan.

Ten hospitals in Australia, including centres in Sydney, Brisbane, Melbourne, Canberra, Adelaide and Perth, have successfully performed Selective Internal Radiation Therapy (SIRT) implants with SIR-Spheres[®].

SIRTeX Medical

SIRTeX Medical Limited has developed three separate cancer therapies based on its micro-particle technologies, which act as transport vehicles to deliver:

- ionising radiation (ie. SIR-Spheres[®], which is used in Selective Internal Radiation Therapy or SIRT);
- chemotherapy drugs (ie Dox-Spheres); and
- materials that can generate heat within the cancer (ie. Thermo-Spheres).

The original concepts underpinning the core technology commercialised by SIRTeX Medical were initially developed by prominent Australian liver surgeon Dr Bruce Gray and the Cancer Research Institute Inc (CRI) and have been published in the scientific literature. The CRI is an independently incorporated research institute, founded in 1990 and has undertaken many research programs investigating cancer over the past decade.

Research and Development

SIR-Spheres[®]

Introduced as an experimental technique in 1987 for treating advanced liver cancer by the Cancer Research Institute in Perth, Western Australia, Selective Internal Radiation Therapy using SIR-Spheres[®] has been refined over many years and has been the subject of several clinical trials.

Two independent clinical trials are currently underway to evaluate the advantages of using SIR-Spheres[®] in combination with two new anti-cancer drugs to improve the treatment of liver cancer. The first trial will test the efficacy of combining SIR-Spheres[®] with an anti-cancer drug combination that includes irinotecan, a major new drug marketed by Pharmacia for the treatment of advanced bowel cancer. As SIR-Spheres[®] is a novel form of radiotherapy proven to regress bowel cancer that has spread to the liver, it is important to evaluate the benefits of adding increasing doses of irinotecan to SIR-Spheres[®]. Supported by Pharmacia, the trial is being conducted in Australia with a small number of patients, and is expected to be completed by the end of 2002.

The second trial will test the use of SIR-Spheres[®] in combination with the drug oxaliplatin - another major anti-cancer drug used for the treatment of advanced bowel cancer. Supported by French-based anti-cancer specialist Sanofi Synthelabo, this trial will be conducted both in Australia and several major cancer centres in Europe.

SIRTeX Medical is also currently in the process of researching and developing Dox-Spheres and Thermo-Spheres. Both are based on the same micro-particle technology as SIR-Spheres[®], but are used to deliver different agents directly to liver tumours.

Dox-Spheres

Current conventional chemotherapy treatments are generally not highly targeted and may have harmful side effects to surrounding healthy tissue. SIRTeX is currently working on incorporating drugs into its microsphere technology to enable the controlled release of the active drug directly to the site of tumours. In the first instance the matrix technology has been developed to transport the drug Doxorubicin (Dox-Spheres). Doxorubicin is a widely used anti-cancer drug that is no longer protected by patents. Dox-Spheres may provide effective treatment for the treatment of solid tumours, particularly tumours in the liver, head and neck.

Thermo-Spheres

Hyperthermia is a technique for destroying cancer cells by raising the temperature of the cancer in the order of 5°C. The subject has been extensively researched over the past 20 years and numerous heating devices based on focused ultrasound or electromagnetic radiation have been produced. None of these devices have been able to accurately deliver high heat loads to deeply situated cancers without also destroying the surrounding normal tissues. The single remaining obstacle to clinical application of hyperthermia is a technological one, rather than a biological one. As the current technology has not overcome the major obstacles for effective heating of cancers, none of the commercially available technologies have gained acceptance and there remains a large potential market for effective hyperthermia technology.

SIRTeX Medical's technology is designed to treat the majority of liver cancer patients that do not have localised tumours and for whom treatment options are limited. Rather than attempting to focus external heat sources from the exterior, SIRTeX Medical has developed a technique whereby small magnetic microspheres (Thermo-Spheres) can be delivered selectively into the cancer and those spheres can then be used to produce localised heating from within the cancer itself.

Company background

SIRTeX Medical was formed in 1997 to acquire and commercialise a portfolio of three technologies relating to the treatment of liver cancer developed by the Cancer Research Institute (CRI) and Dr Bruce Gray. At that time, NJI No. 2 Investment Fund (managed by Nomura/JAFCO Investment (Asia) Ltd) subscribed for equity in the Company to finance development of the technologies. Since its formation, SIRTeX Medical has both undertaken its own research and development and also contracted other agencies such as the CRI to further develop the Company's technologies.

SIRTeX Medical aims to become the world leader in liver cancer treatment products. The company believes the unmet demand for effective treatment of liver cancer provides an opportunity for SIRTeX Medical's products. SIRTeX will also apply its microsphere technology to other diseases where the Company's intellectual property can provide market opportunities.

For further information, please see our website at www.sirtex.com.

France-Australia Symposium on Nuclear Medicine

Sydney, Australia April 29, 2002

Poster List

1. Exploration of the dopamine transporter (DAT): from Chemistry to Clinical application

P. Emond, S. Chalon, F. Dollé, C. Halldin, D. Guilloteau
INSERM U 316, CHU Bretonneau, Université François Rabelais Tours France
Service Hospitalier Frédéric Joliot, DRM, CEA Orsay France
Karolinska Institut, Department of Clinical Neuroscience, Stockholm, Sweden.

2. Pharmacological characterization of MADAM: a potent and selective ligand for the serotonin transporter

D. Guilloteau, J. Tarkiainen, P. Emond, H. Hall, L. Garreau, J. Vercouillie, J. Sandell, JC Besnard, C. Halldin
S. Chalon
INSERM U 316, CHU Bretonneau, Université François Rabelais Tours France
Karolinska Institut, Department of Clinical Neuroscience, Stockholm, Sweden

3. Biodistribution and radiation dosimetry of the nicotinic ligand [¹⁸F]fluoro-A85380 in healthy volunteers.

H. Valette, M. Bottlaender, D. Roumenov, F. Dollé, C. Coulon, M. Ottaviani, M. Ricard, and A. Syrota. CEA, Service Hospitalier Frédéric Joliot, DSV/DRM, 91406 Orsay, France; § Service de Physique, Institut Gustave Roussy, 94805 Villejuif, France.

4. Design, Synthesis and Pharmacological Characterization of New Highly Selective Corticotropin-Releasing Factor, Type 2 (CRFR2) Antagonists.

A. Ruhmann, J. Chapman, J. Higelin, and F.M. Dautzenberg. Radiopharmaceuticals Division R&D, ANSTO, F. Hoffmann-La Roche Ltd., Pharma Division, Preclinical Research, Basel, Switzerland

5. Chelating agents and vector technology for application in Nuclear Medicine And Environment

A. Loussuarn CHELATEC SAS, Nantes, France.

6. Animage: new approaches for functional exploration of small animals.

Didier Le Bars CERMEP Lyon

7. Synthesis of Silica Nanoparticles for Controlled Drug Release

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8. International Nuclear Information System (INIS) at ANSTO

M. Huxlin. Information Management Division, ANSTO

9. Synthesis And Enantiomeric Evaluation Of Radiolabelled Methyl Phenidate For The Study Of Dopamine Reuptake Sites

A. Katsifis, F. Mattner, K. Mardon, B. Dikic, V. Papazian, T. Jackson, I. Greguric.
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10. Synthesis And Evaluation Of Radioiodinated Ligands For The Study Of Peripheral Benzodiazepine Receptors Using Spect

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Poster 3

"Biodistribution and radiation dosimetry of the nicotinic ligand [^{18}F]fluoro-A-85380 in healthy volunteers"

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INTRODUCTION

General goal: development of 2- [^{18}F]fluoro-3-[2(S)-2-azetidylmethoxy]pyridine ([^{18}F]A-85380) a radiolabeled tracer suitable for imaging $\alpha 4\beta 2$ subtype neuronal acetylcholine receptors.

Specific goal: As part of Phase I studies, assessment the whole-body biodistribution of [^{18}F]A-85380 and calculation of associated radiation-absorbed doses in healthy human volunteers.

MATERIALS AND METHODS

SUBJECTS 3 healthy male volunteers (age: 22 years, weight: 74 kg); Non-smoker (negative plasma cotinine determination); Serum chemical analysis, complete blood cell count and urine analysis: before and within a week after completion of the PET study.; 24 venous blood samples during the emission scan. Urine collection at the end of emission scan. **PET IMAGING PROCEDURE** Whole-body scans on the ECAT Exact HR+ PET scanner. Transmission: three [^{68}Ge] retractable rods (2mCi per rod). 3min per bed position (FOV: 15cm), with 8 or 9 steps. Emission: One minute step acquisition, 8 or 9 steps for one scan (top the head to the upper part of the thigh) for 2 hours after iv injection of 191 ± 50.9 MBq i.e. 1.73 ± 0.21 nmoles [^{18}F]A85380.

PET DATA ANALYSIS Reconstruction: OSEM algorithm (4 iterations and 8 subsets) in the transaxial format with segmented attenuation correction. 2D views assembled with bed position overlap (12 tomographic slices) and smoothed with a gaussian kernel 5mm FWHM. ROIs: whole brain, liver, intestines (upper and lower large intestine wall), stomach, spleen, kidneys and bladder. Dosimetry analysis: construction of time activity curves (TAC) using whole-organ activities at the scan time. *Residence times*: division of the area under the TACs by the injected dose. Source organ residence times: determined from integration of the TACs from the best fit obtained in the compartmental model.

RESULTS

Biosafety: No adverse or subjective effects were noticed. No significant change in heart rate, blood pressure, ECG findings were observed during the 10 min following injection of [^{18}F]A85380. No meaningful changes in any of the clinical laboratory assays performed on the blood and urine specimens. **BIODISTRIBUTION AND DOSIMETRY** Penetration of [^{18}F]A-85380 across the blood brain barrier peaked at 50 min and was followed by a slow clearance. Brain: average % I.D. was 0.182/g tissue (range: 0.16-0.196 % I.D./g tissue); Blood: Average percentage of injected dose remaining in the blood 60min post injection was 0.055 (range: 0.05-0.06 %I.D./g tissue). Urine: mean measured excretion at 2 h post-injection: 20% injected dose.

Mean effective dose equivalents: 0.0194 mSv/MBq (range: 0.0178-0.0218 mSv/MBq).

DISCUSSION

Favorable biodistribution of [^{18}F]A-85380 in human volunteers with approximately 0.2% I.D./gram distributing to the brain. Pharmacologically safe radioligand; Main route of elimination: the renal system, The amount of [^{18}F]A85380 in the liver and kidneys was initially high (1% I.D. /g tissue), but the clearance in these organs was rapid. The calculated radiation absorbed dose estimates remained well within the range of doses acceptable in clinical nuclear medicine studies. 185 MBq [^{18}F]A-85380 gives an average effective dose equivalent of approximately 4.3 mSv.

CONCLUSION

Based on the average effective dose equivalent of 0.0194 mSv/MBq, both patients and human volunteers could be investigated with a multi-injection protocol for the quantification of central nAChRs.

Residence times (hours) for [^{18}F] A85380 for each source organ:

Source organs	Subject 1	Subject 2	Subject 3	Mean $\pm$ SD
Liver	0.268	0.205	0.28	0.251 $\pm$ 0.04
Spleen	0.014	0.011	0.013	0.013 $\pm$ 0.002
Brain	0.062	0.053	0.069	0.061 $\pm$ 0.008
Red bone marrow	0.25	0.15	0.146	0.182 $\pm$ 0.059
Kidneys	0.07	0.05	0.062	0.061 $\pm$ 0.01
Urinary Bladder	0.108	0.267	0.072	0.149 $\pm$ 0.1
Small intestine	0.118	0.06	0.053	0.077 $\pm$ 0.036
ULI	0.065	0.04	0.037	0.047 $\pm$ 0.015
LLI	0.04	0.031	0.029	0.033 $\pm$ 0.006
Remainder	1.49	1.635	1.87	1.665 $\pm$ 0.194

Nuclear Medicine Environment

CHELATION (Complexation)

CUSTOMIZED RADIOLABELING

VECTORIZATION

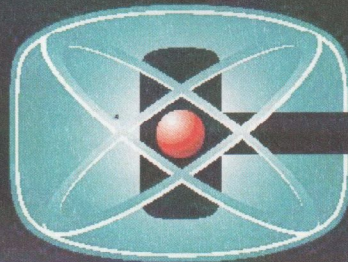
PHARMACOKINETICS

BIODISTRIBUTION

DECORPORATION

PURIFICATION

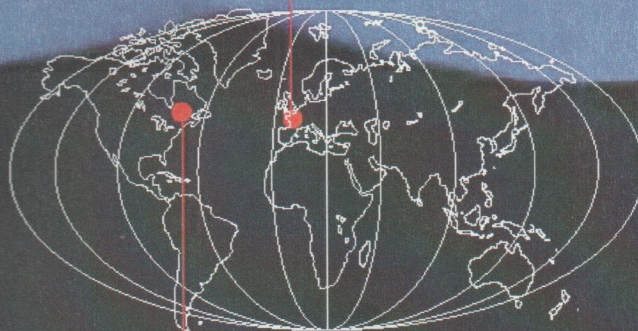
EXTRACTION



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Poster 7

SOL-GEL MICROSPHERES AND NANOSPHERES FOR CONTROLLED RELEASE APPLICATIONS

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ABSTRACT SUMMARY

We present a novel approach to the synthesis of inorganic sol-gel microspheres for encapsulating organic and bioactive molecules, and controlling their subsequent release kinetics. The bioactive species are incorporated, at ambient temperature, into the inorganic particles using an emulsion gelation process. Independent control of the release rate (by adapting the nanostructure of the internal pore network to the physico-chemical properties of the bioactive molecules) and particle size (by tailoring the emulsion chemistry) is demonstrated.

Keywords: ceramics, encapsulation, inorganic gels, hydrophilic matrices, microspheres

INTRODUCTION

Sol-gel chemistry has been shown to be a flexible technique for producing inorganic silica matrices with tailored microstructures, which can be used for the encapsulation and controlled release of organic and bioactive molecules. The present paper extends this concept by combining sol-gel chemistry with an emulsion approach for producing inorganic particles with controlled dimensions, and demonstrates how the particle size and microstructure can be independently controlled.

EXPERIMENTAL METHODS

Sol-Gel Chemistry and Encapsulation of Model Compounds. A stock solution of 4-(2-hydroxy-1-naphthylazo) benzene sulfonic acid (*Orange II*) was produced by dissolving *Orange II* in water (0.1 wt%), and adjusting the pH to the required value. Sol-gel solutions were subsequently prepared by mixing the aqueous solution with tetramethylorthosilicate (TMOS) and methanol (MeOH), to achieve H₂O:TMOS (*W*) and MeOH:TMOS mole ratios (*D*) of four. The resulting solution was stirred and left to age at ambient temperature for one day.

A transparent emulsion was prepared by mixing selected surfactants and organic solvents. The surfactants used included sorbitan monooleate, sorbitan monolaurate and bis-2-ethylhexylsulfosuccinate (AOT), while the organic phase was typically chosen from the group consisting of

kerosene, hexane, heptane, octane, decane, dodecane and cyclohexane. The sol-gel solution was added to the emulsion, and the resulting mixture was stirred at 500 rpm for one hour. The particles thus produced were washed with cyclohexane and carefully dried.

Release Kinetics. The release of the encapsulated species from the particles was monitored by placing a fixed quantity of the sample in water or physiological saline, and monitoring the concentration of the released species using UV/Vis spectroscopy. The fraction released was normalised to the total mass initially encapsulated.

RESULTS AND DISCUSSION

1. Influence of emulsion chemistry on particle size.

The influence of the emulsion chemistry on the size of the microspheres obtained at pH 2 is illustrated in Figure 1 for microspheres containing encapsulated *Orange II*. Using essentially identical sol-gel chemistries, particles with dimensions ranging from 50 nm to 50 μ m can be readily obtained.

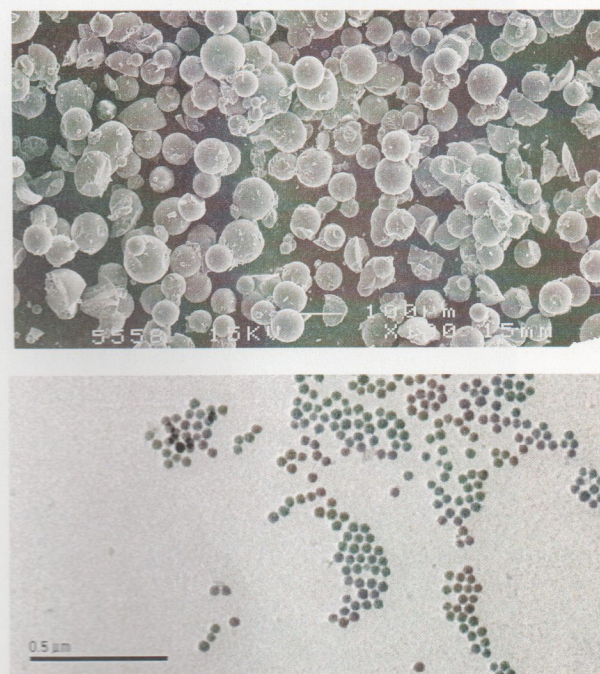


Figure 1: Encapsulation of *Orange II* in silica sol-gel nanospheres and microspheres obtained using heptane/sorbitan monooleate (TOP) and cyclohexane/NP9 (BOTTOM).

The size of the particles is controlled by the size of the emulsion droplets, which act as micro-reactors for the sol-gel reactions. In turn, the size of the droplets is determined predominantly by the hydrophile/lipophile balance of the surfactant/solvent couple and by processing parameters such as the water to surfactant ratio and the concentration of surfactant.

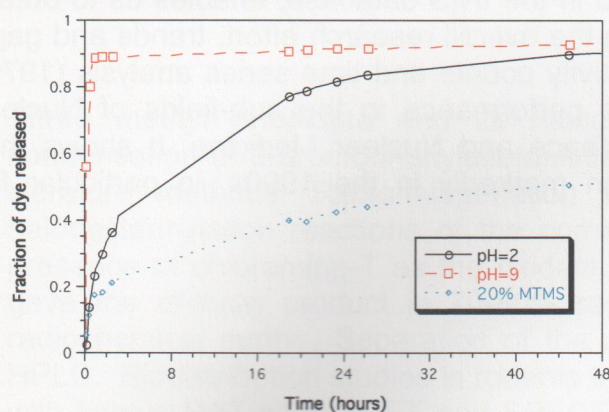


Figure 2: Effect of sol-gel synthesis conditions on the microstructure and release kinetics. Samples illustrated in the **TOP** and **BOTTOM** micrographs were prepared at pH 2 and 11, respectively, using sorbitan monooleate and kerosene. The corresponding kinetics for the release of encapsulated *Orange II* are shown in the **BOTTOM** traces.

2. Influence of Sol-Gel Solution Chemistry on Release Rates.

The effect of sol-gel processing parameters on the morphology and associated release kinetics is illustrated in Figure 2. Although both samples have essentially identical particle sizes (not shown), their morphologies are significantly different. Processing under acidic conditions leads to the formation of nanosized linear silicate "building block" which are woven tightly together in the final gel matrix, to produce a microporous material (pore diameters < 2 nm). In contrast, three-dimensional fundamental particles are produced under basic conditions, leading to the formation of mesoporous gels (pore diameters > 2 nm). The different morphologies are clearly reflected in the release rate of the encapsulated species from the respective matrices; rapid release kinetics are evident for the mesoporous material (high pH), while significantly slower release rates are obtained for the microporous material (low pH).

CONCLUSIONS

A combined sol-gel/emulsion processing method has been developed to produce inorganic matrices with tailored nanostructures containing encapsulated organic and bioactive molecules. Control of the release kinetics is achieved by tailoring the internal nanostructure of the matrix during synthesis and encapsulation, while the particle size of the matrices is controlled through the emulsion chemistry. This approach enables the size and internal structure of the matrices to be independently controlled. The wide range of particle size (from nanometers to microns), combined with the potential to control the release kinetics independently from the particle size, make this technology very attractive for a wide range of therapeutic applications.

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Poster 8

International Nuclear Information System (INIS) at ANSTO

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INIS is the world-leading information system in the field of nuclear science and technology. It is operated by the International Atomic Energy Agency (IAEA) in collaboration with 103 Member States and 19 international organisations. It contains over 2 million bibliographic references (1970-present) and a unique collection of scientific and technical reports, conference papers, dissertations, patents and others documents, known as the "grey literature". ANSTO hosts the Australian INIS Centre, which is responsible for the collection and processing of the Australian material for inclusion in the database as well as dissemination of INIS output products in Australia. Through its participation in INIS Australia gains access to the result of billions of dollars of nuclear-related R&D from around the world, and promote nuclear scientific and technical developments in Australia to the international science community.

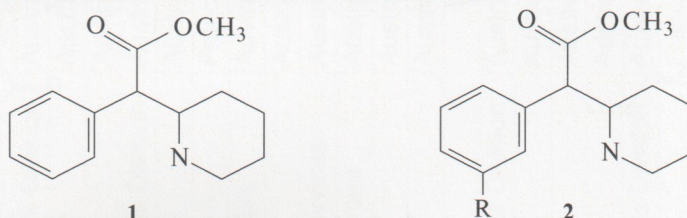
A particular case is presented, which illustrates how INIS could be used to evaluate the research effort in nuclear science and technology. Citation analysis, usually based on journals indexed by Institute for Scientific Information, measures the impact of the research or rather the usefulness of research to other scientists doing related work. However, a bibliometric analysis of this kind will not be representative of the whole research effort in the field of nuclear science and technology where a relatively high proportion of the output (45%) is captured in the non-journal literature. Publication counts based upon all publications indexed in the INIS database, enables us to obtain statistics and scientific indicators regarding the overall research effort, trends and gaps within this particular field. Average productivity counts and time series analysis (1976-2000) give a glimpse into the Australia's performance in the sub-fields of Nuclear Chemistry, Nuclear Physics, Materials Science and Nuclear Medicine. It shows that Australia's share of publications has risen markedly in the 1990s, in particular for Materials Science and Nuclear Medicine.

Poster 9

Synthesis and Enantiomeric Evaluation of Radiolabelled Methyl Phenidate for the Study of Dopamine Reuptake Sites

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Neurological diseases such as Parkinson's Disease and associated Movement Disorders have been characterised by reductions in the number of dopamine re-uptake sites or dopamine transporters (DAT) on presynaptic neurons in the striatum. Radiolabelled drugs which display specific and selective binding to the DAT has thus found clinical utility in medical imaging. The psychomotor stimulant methyl phenidate (MP) or Ritalin **1** and several derivatives have demonstrated high affinity binding to these DAT where they exert their pharmacological action. MP possesses two chiral centers with its pharmacological activity being attributed to the *dl-threo* diastereomer, the *dl-erythro* being inactive. Furthermore, imaging studies using Positron Emission Tomography (PET) with carbon-11 radiolabelled MP confirmed the *d-threo* enantiomer to be most active. *In vitro* studies indicate that compounds substituted with either bromine or iodine in the 3 position of the aromatic ring exhibit high affinity and selective binding to DAT and were thus targeted for radiolabelling with both the PET tracer Bromine-76 as well as the SPECT tracer Iodine-123.



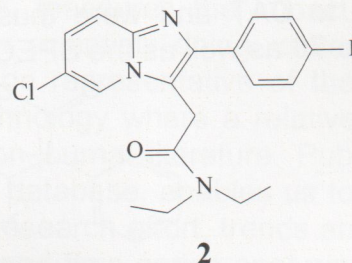
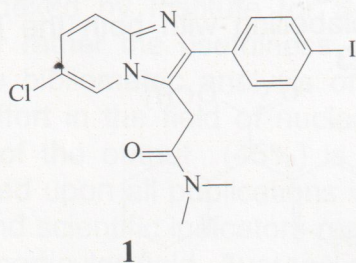
Threo methyl phenidate and its halogenated derivatives were prepared by the condensation of 3-bromophenylacetonitrile and 2-bromopyridine according to modified literature methods. Radiohalogenation with either ^{76}Br or ^{123}I was achieved by halodestannylation reactions of the corresponding tributyl stannane precursor in the presence of chloramine-T as the oxidant. Purification by C-18 reverse phase HPLC gave the *dl-threo* product in 70-80% radiochemical yield and in greater than 95% radiochemical purity. Separation of the *d* and *l* enantiomers was achieved by chiral HPLC. Biodistribution studies in rodents indicated high uptake of radioactivity in tissues with known DAT sites. PET and SPECT imaging studies in primates indicated high uptake in the striatum compared to the cerebellum reference tissue. The synthesis, diastereomeric separation, structure activity studies, radiolabelling and biological studies of these compounds will be presented.

Poster 10

Synthesis and Evaluation of Radioiodinated Ligands for the Study of Peripheral Benzodiazepine Receptors Using Spect

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The peripheral benzodiazepine receptor (PBR) is a multimeric protein complex located in the outer mitochondrial membrane and predominantly found in steroid producing organs and glial cells in the brain. The PBR have been implicated in the control of cell proliferation and differentiation and shown to display increased levels in a variety of malignant tumours and neurodegenerative disorders. A series of potent imidazo[1,2-a]pyridines have been prepared for development as radiopharmaceuticals to study these disorders in patients using nuclear medicine imaging techniques. *In vitro* studies indicate that compounds substituted with an electronegative atom in the 6 position of the pyridine ring, a lipophilic group or halogen in the 4'-position of the 2-phenyl ring, and lower alkyl methyl or ethyl substituents on the amide nitrogens of the side chain, exhibit high affinity and selective binding.



N,N'-dimethyl- and the N,N'-diethyl 6-chloro-(4'-iodophenyl)imidazo[1,2-a]pyridine-3-acetamide **1** and **2** displayed optimum *in vitro* properties and were thus selected for radiolabelling with the diagnostic radionuclide iodine-123. Radioiodination was achieved by iododestannylation of the corresponding tributyl stannane precursor in the presence of peracetic acid. Purification by C-18 reverse phase HPLC gave the desired products in 70-80% radiochemical yields and in greater than 98% radiochemical purity. Biodistribution studies in normal rodents indicated high uptake of radioactivity in tissues with known PBR sites. Preliminary imaging studies in rodents bearing mammary adenocarcinomas indicated high uptake in the tumour with retention of activity after 24 h.

The synthesis, structure activity studies, radiolabelling and biological studies of these compounds will be presented.

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