by Stephen J. Mather

NEW HORIZONS IN RADIOPHARMACEUTICALS

Nuclear Medicine must concentrate on its unique strengths if it is to continue to prove clinically useful and survive into the new century.

People undertake research in radiopharmacy for many different reasons. Those that work in the pharmaceutical industry follow defined development strategies with the ultimate aim of registering a new product and generating money for the company. This article is concerned mostly with the work that is conducted in academic institutions where intellectual, rather than financial, pursuits are the norm. The most successful research projects are normally those that seek to solve a particular clinical problem. This provides the necessary focus to the project as well as giving the researchers the ultimate satisfaction of seeing their endeavours put to good use. With the improvements in competing modalities – such as spiral CT, Doppler echo and spectroscopic MRI for determination of locality and perfusion of disease – there is an increasing need for Nuclear Medicine to concentrate on its unique ability to perform functional assessment of tissues and, in particular, to try and assess intracellular as well as extracellular changes. Recent developments in the field of targeted radionuclide therapy have also given a fresh impetus to work in this area.

This review presents the authors' personal view of some of the most promising areas of current radiopharmaceutical research. These can be summarised as:

- Infection imaging;
- Cancer imaging;
- Cancer therapy;
- Neuro-receptor imaging; and
- Radiopharmaceutical chemistry.

Infection imaging is perhaps the most prolific area for radiopharmaceutical development and a survey of the literature would surely result in dozens of potentially 'useful' new products, but few, if any, have stood the test of time. The main challenges in infection imaging are the ability to distinguish true infection from sterile inflammatory processes and the need for a universal detector of inflammation to replace the use of radiolabelled white cells. In cancer, the diagnostic applications of Nuclear Medicine have moved away from early screening and primary diagnosis towards secondary staging and subsequent individual tailoring of patient therapies. As new expensive biological therapeutic possibilities become available, it will be essential to determine ways of identifying those patients who will benefit from such treatment.

For the first time in many years new therapeutic radiopharmaceuticals – such as labelled anti-CD20 antibodies in lymphoma, radiolabelled octreotide analogs in neuroendocrine disease, and radiolabelled phosphonates for bone metastases – are proving to have real clinical utility. This has stimulated further research into other therapeutic applications and even the use of 'new' radionuclides such as the beta-emitter Lu-177 and alpha-emitters such as Bi-213.

The challenge in neuroreceptor imaging is to translate the successes of the PET field into SPECT tracers. Although a number of ^{99m}Tc-labelled ligands, which bind to receptors *in vitro*, have been developed, their application *in-vivo* continues to be limited by the low brain uptake caused by the sub-optimal physico-chemical properties of these compounds.

Progress in radiopharmaceutical chemistry, such as the development of the aqueous route to the tricarbonyl technetium-99m precursor, continues to produce new complexes with novel properties that will provide unexplored avenues for clinical exploitation in years to come.

Since its inception nearly fifty or so years ago, radio-pharmaceutical development has proceeded through a number of phases. The early years, 1950's to mid-60's, were characterised by the clinical application of naturally occurring radioactive salts such as ^{131}I-iodide and ^{32}P phosphate and, following the introduction of technetium-99m, by developments thereof such as ^{99m}Tc-pyrophosphate for bone scanning. The period up to the early 80's pursued the aim of developing radio-pharmaceuticals that were taken up by the major organs of the body by a variety of different mechanisms – colloids for liver scanning, macroaggregates for lung scanning, ^{99m}Tc-DTPA and DMSA for kidney imaging etc. The 80's were the years of the application of technetium coordination chemistry and resulted in a number of tracers that measure regional organ perfusion or function: ^{99m}Tc-exametazime for brain perfusion, ^{99m}Tc-sestamibi and tetrofosmin for myocardial perfusion and ^{99m}Tc-MAG3 for renal tubular secretion.

In the 90's, a shift occurred towards imaging the characteristics of groups of cells rather than whole organs. Radiolabelled monoclonal antibodies

targeting tumour associated epitopes and neuropeptides such as somatostatin analogues were developed for imaging the over-expression of their receptors on the surface of malignant cells.

This trend has continued in this millennium and is likely to do so for the foreseeable future. The increasing availability of strong competing imaging modalities such as MRI and spiral-CT means that Nuclear Medicine must concentrate on its unique strengths if it is to continue to prove clinically useful and hence survive into the new century.

The two most important attributes of Nuclear Medicine are (i) the use of very high specific activity tracers which permit the possibility of imaging low capacity mechanisms *in-vivo* and (ii) the therapeutic application of targeted radionuclides. The most successful radiopharmaceutical developments in the future will arise from the application of these strengths to the solution of real clinical problems. Thus the directions of radiopharmaceutical research will tend to move from localisation of disease to functional assessment of tissues, from targets on the outside of cell membranes to those buried deep within the cytoplasm and cell nucleus, from 'passive' imaging in the Nuclear Medicine department to interventional applications in the operating theatre and from the diagnosis of disease to its treatment.

These developments are likely to occur mainly in the following areas of research:

- Inflammation imaging;
- Cancer imaging;
- Cancer therapy;
- Neuroreceptor imaging;
- Radiopharmaceutical chemistry

Inflammation imaging

The issues in inflammation imaging lie in the complexity of the current gold standard investigation – labelled white cells, and also in its inability to distinguish between inflammation caused by underlying infection from that caused by other causes. The widely used practice of blood labelling is time consuming, requires special skills and facilities and carries the risk of needle-stick infection from blood-borne infections such as hepatitis and HIV. Attempts to overcome this problem have included the use of tracers that label white cells *in-vivo* in whole-blood thereby removing the need for manipulation of the blood *ex-vivo*. Perhaps the most widely used example is Leukoscan^{TM} (^{99m}Tc-Sulesomab), a radiolabelled antibody fragment which binds to the NCA-90 epitope on white cells. Although developed with the idea that the antibody would bind to circulating white cells which subsequently migrate to the site of infection, it appears that this is not its real mechanism of action. The uptake is due in part to 'non-specific' extravasation of the labelled antibody at the site of infection followed (perhaps) by binding to local white cells in the vicinity. Other ligands, such as cytokines and chemotactic peptides, which recognise different markers on different populations of white cells, are currently being explored and may find wider application in the future. However, the knowledge that non-specific mechanisms can contribute to imaging of inflammation has led several research groups to pursue entirely non-white cell mediated solutions to this problem. Among the most successful has been the use of non-specific immunoglobulins (HIG) and liposomes. These can be labelled with a variety of radioisotopes, in particular technetium-99m and indium-111, and therefore have the potential for use in imaging both the same day and for several days after administration. Clinical trials with these agents have demonstrated high sensitivity in the detection of inflammation but neither has achieved widespread use. Perhaps the main cause for this is that, for a variety of reasons, no commercial manufacturer has developed the product, obtained market authorisation and made it universally available. Without this commercial development any radiopharmaceutical, however 'good', is likely to remain an item of purely academic interest.

The second important issue in inflammation imaging is identifying the source of the inflammation. The important question is whether or not to continue the use of antibiotics. Thus attempts are being made to develop radiopharmaceuticals that interact not with the body's own defence mechanisms but with the invading micro-organisms themselves. Among the current candidates of interest are the defensins – naturally occurring peptides that bind to a broad spectrum of bacteria. Although these have been shown to have some degree of specificity for infection rather than sterile inflammation, the target to background ratios achieved have been relatively modest and their use has not yet been pursued in the clinic. By contrast, the use of ^{99m}Tc labelled ciprofloxacin, a fluoroquinolone antibiotic, has been studied in almost 1,000 patients with encouraging results. In fact, ciprofloxacin is only one of a significant number of antibiotics that have been labelled with the aim of infection imaging. More developments can be expected in this field as antibiotics, with more specific bacterial interactions and more favourable patterns of biodistribution, are identified. Of particular interest is the use of these drugs for imaging non-bacterial infections. Antibiotics specific for fungal or parasitic infections may be very valuable in the context of adventitious infections in immuno-compromised patients or in the developing world.

Cancer imaging

The areas of possible application of radiopharmaceuticals in the management of patients with cancer include:

- Population screening;
- Primary diagnosis;
- Staging of disease;
- Measuring response to therapy;
- Tailoring and identifying optimal therapies.

The reality is that socio-economic issues, as well as clinical realities, mean that Nuclear Medicine is unlikely to play a significant role in either screening or primary diagnosis. But it can play an increasingly important part in all three of the subsequent areas of management.

Staging in cancer requires an imaging investigation which provides rapid throughput, whole-body imaging, high sensitivity and high specificity. In recent years it has been recognised that ^{18}FDG can deliver at least the first two of these attributes and clinical PET centre development is now the most rapidly developing application of Nuclear Medicine in the developed world. At the moment, another radiopharmaceutical able to compete with FDG in this arena is not on the horizon. New developments are therefore likely to be directed towards producing complementary tracers that can help to overcome the potentially limited

specificity of FDG. Among the most widely explored approaches are the uses of radiolabelled neuropeptides. Although their application is normally limited to those specific diseases in which expression of the receptors is elevated, these radiopharmaceuticals do have the potential to fill this current deficiency in cancer staging. To date, the most widely explored field of peripheral neuroreceptor imaging remains the family of somatostatin receptors. However, because of its success, this application is also encouraging the development of new radiolabelling technology which not only improves the performance of somatostatin receptor imaging, but will have broader utility across the field of neuropeptide receptor targeting. Examples include the development of improved methods for labelling peptides with technetium-99m. Thus, the combination of the use of hydrazinonicotinamide (HYNIC) with a variety of co-ligands for technetium coordination has been shown to have a profound influence on the performance of these tracers for imaging and the development of a simple method for producing the tri-carbonyl, tri-aqua reactive technetium intermediate provides the opportunity for producing new peptide complexes with novel imaging characteristics. The horizon will see the application of this new chemistry to a range of ligands binding other neuropeptide receptors such as those for neurotensin, gastrin, gastrin-releasing peptide, and vasoactive intestinal peptide.

Many well-established treatments for cancer are highly toxic and one of the major deficiencies in the current management of patients is our inability to identify whether individual patients will benefit from a particular combination of drugs. The classical measure of tumour shrinkage is normally only able to provide information some time after the patient has received a course of often debilitating therapy. An investigation which could provide information on the effectiveness of a particular therapy, even after one dose, could be very valuable and save not only a lot of money but also a lot of unnecessary toxicity by patients who will not benefit significantly from their treatment. One of the most valuable measures of drug response would be its effect on tumour cell proliferation. In order to try and image this process, researchers have labelled a variety of substrates for cell metabolism including a number of different nucleotides and amino-acids. One of the most widely studied is ^{18}F-3'-deoxy-3'-fluorothymidine (FLT). PET imaging with FLT delineates the major sites of normal cell proliferation, in particular the bone marrow, and FLT is also able to efficiently image many tumours albeit with rather lower SUV values than FDG. FLT is likely to be just one of the forerunners of a new family of radiopharmaceuticals for imaging proliferation which, when administered before and after a cytotoxic drug, would be able to provide a quantitative measure of drug response. However, to justify the clinical use of such tracers, it is important to validate their mechanism of uptake and retention and FLT represents a good model for such studies. FLT diffuses across the cell membrane into the cytosol where it is trapped following phosphorylation by thymidine kinase$_1$. Thus, the uptake of FLT by a tissue will depend upon the level of TK_1 activity therein. In order to determine if TK_1 activity is related to proliferation, Rasey and co-workers (Rasey J.S, Grierson J.R, Wiens L.W, Kolb P.D, Schwarz J.L., paper in *Journal of Nuclear Medicine*, 2002; 43: 1210-7) compared the cellular uptake of FLT with the number of dividing cells and the TK1 level and found a linear relationship with both parameters. From this one could conclude that FLT uptake is indeed a measure of proliferation. However, in a transformed cell with an increased appetite for metabolic substrates such as thymidine, situations may also arise in which TK_1 levels are elevated independently of proliferation since this would provide a survival benefit. Wagner and co-workers (Seitz U., Wagner M., Neumaier B., Wawra E., Glatting G., Leder G., et al., paper in *European Journal of Nuclear Medicine Molecular Imaging*, 2002; 29: 1174-81) studied the levels of TK_1 (and other enzyme) activity is a variety of pancreatic cell-lines and found that the TK_1 levels varied independently of cell proliferation. Many such studies will be performed over the next few years to validate the use of markers of proliferation before their routine clinical application can be established.

One of the mechanisms by which cancer treatments mediate their effect is by programmed cell death or apoptosis. This arises when cell surveillance systems detect high levels of DNA damage which, if the cell was allowed to divide normally, would lead to a risk of inherited mutations in the genetic code. One of the effects of apoptosis is a refolding of the cell membrane resulting in the exposure on the outside of the cell of elements which are normally on the inner surface. These elements can be used as targets for radiopharmaceuticals which aim to image the apoptotic process as a measure of tumour response to therapy. The most well developed approach is the use of radiolabelled annexin-V. Annexin-V binds to phosphatidylserine which is one of the cell membrane components exposed during the early stages of apoptosis. Annexin-V has been labelled with technetium-99m and studied in a number of clinical trials in which apoptosis is expected to occur. Although originally developed as a marker of tumour response and validated in animal models, in fact some of the most impressive images have been acquired in 'natural' apoptosis such as, in myocardial infarction. Although uptake of ^{99m}Tc-annexin-V has been seen in tumours following cytotoxic or radiotherapy, the degree of uptake is very varied and the quality of the images not impressive. There are certainly a number of reasons for this; not least that apoptosis is a transient phenomenon the timing of which is variable and unpredictable. However a contributory factor is that annexin-V itself is not an ideal substrate for radiopharmaceutical application. It is a relatively large protein that clears only slowly from the blood and, like other proteins, diffuses only slowly into tumours. The target to background ratios that are achieved are therefore sub-optimal. It seems likely that annexin-V will be a paradigm for a new generation of radiopharmaceuticals for imaging apoptosis that have more ideal pharmacokinetic characteristics.

While these markers of response relate primarily to conventional established cancer therapies, radiopharmaceuticals also have a potential use in defining the role of newly emerging therapies. For example anti-angiogenic therapies have little immediate effect on tumour size, and their effectiveness cannot be measured using conventional structural imaging. Tracers, which target VEGF and other markers expressed during angiogenesis, could be used as surrogate

markers of drug efficacy in clinical trials of these compounds.

As well as indicating responses to therapy in cancer, radiopharmaceuticals may also help to identify those patients which will benefit from a particular type of therapy. It has long been known that tumours, which are hypoxic, are less responsive to external beam radiation therapy than those with normal oxygen levels. This has prompted a search for radiopharmaceuticals — the uptake of which is determined by tissue oxygen levels. The most widely explored class of compounds are the nitroimidazoles. These are preferentially reduced and consequently bound in hypoxic tissues and, if radiolabelled, can potentially be used to image the distribution of oxygen tension in tumours and other important organs such as the heart. Nitroimidazoles have been labelled with a variety of radionuclides including fluorine-18 (F-MISO) and iodine-123 (IAZA) as well as with technetium-99m. Several of these are in clinical trials although their role, and the relationships between uptake and other markers of oxygen tension, has yet to be established. A new class of copper-based radiopharmaceuticals, which show varying degrees of retention in hypoxic or normoxic tissues, has recently emerged. The mechanism of retention of these N4 semithiocarbazone tracers is reduction of Cu(II) to Cu(I) followed by loss of the radiometal from the complex. It has been shown that varying the substituents on the periphery of the complex can change the reduction potential of the copper core and by suitable adjustment, a complex which is retained by all normoxic cells, e.g. Cu-ATSM, or only by hypoxic cells, e.g. Cu-PTSM, can therefore be designed.

Cancer therapy

As suggested at the beginning of this review, one of the unique strengths of Nuclear Medicine is the possibility of targeted radionuclide therapy and recent years have seen a resurgence of interest in this field. The stimulus for this has been the development of two new indications for targeted therapy – radiolabelled anti-CD20 antibodies for therapy of lymphoma and radiolabelled octreotide analogues for treatment of neuroendocrine cancer. A major difficulty in this area of research is the lack of basic knowledge on what are the important determinants of effective therapy. We employ a variety of different radionuclides with a range of different physical decay properties without a real understanding of what the optimal half-life, type and energy of particulate emission are. Nor do we know much about the mechanism of action of low-dose targeted radiotherapy. Is it the same as high-dose external beam therapy, i.e. primarily double-stranded DNA cleavage, or do different mechanisms dominate? More basic research is needed to define these parameters. At the same time, it has been argued that irrespective of the underlying mechanisms, the most important type of studies are *in-vivo* trials of efficacy either in patients or animal models. For such studies, the most overriding concern is often the availability of the radioisotope rather than its mode of decay. In recent years the number of commercially available radionuclides has increased with several sources of yttrium-90 and, more recently, lutetium-177 appearing. This is likely to lead to a significant increase in the ability of researchers to undertake clinical trials in this arena.

Neuroreceptor imaging

For many years the development of radiopharmaceuticals for imaging neuroreceptors in the brain has been the preserve of specialist PET chemists. As a consequence of their work, a large number of radioligands with affinity for a variety of receptor types and subtypes, have been developed but the application of these tracers has been limited to a relatively few specialised centres. If this type of radiopharmaceutical is to become more widely used then, either more centres will need to develop access to the technology (a real likelihood with the development of clinical PET) or analogues of these tracers labelled with single photon emitting radionuclides need to be developed. A significant number of iodine-123 labelled compounds has now been established and at least one (^{123}I-FP-CIT or DatScan^{TM}) is now approved for general use, but the expense and limited availability of iodine-123 remain a problem. The development of technetium-99m labelled receptor ligands is still the goal of several research groups but its fulfilment remains elusive. A useful neuroreceptor imaging radiopharmaceutical must show high stability (in solution, in serum and *in-vitro*), high *in-vitro* binding affinity, good in-vitro binding selectivity, acceptable brain uptake and specific receptor uptake *in-vivo*. While the first three requirements are achievable, combining these with a sufficient degree of brain uptake presents a real problem. To date, effective tracers have only been developed for one target, the dopamine transporter. It is expected that more lie ahead.

Summary

The underpinning technology, which links all of the clinical applications described in this review, is radiopharmaceutical chemistry. An understanding of the coordination chemistry, which allows the preparation of stable complexes, and an appreciation of the relationships between this chemistry and the behaviour of the radiotracers in biological environments, is essential if developments in radiopharmaceutical design are to continue. The ability to manipulate the stability, charge, size and lipophilicity of bifunctional chelates in particular will allow us to generate new complexes with novel physicochemical properties that translate into novel patterns of biodistributions and thus provide a new generation of radiopharmaceuticals beyond the horizon.

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