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Observations on the Role of Nuclear Medicine in Molecular Imaging

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Abstract The phrase ''molecular imaging'' is unquestionably current and is receiving ever increasing use. For example, two organizations, the Institute for Molecular Imaging and the Academy of Molecular Imaging have recently been established with molecular imaging as their focus, with journal entitled ''Molecular Imaging'' and ''Molecular Imaging and Biology,'' respectively. Furthermore, the two leading journals in the field of nuclear medicine have recently added this phrase to their covers-becoming the ''European Journal of Nuclear Medicine and Molecular Imaging'' and ''The Journal of Nuclear Medicine—advancing molecular imaging.'' The National Institute of Biomedical Imaging and Bioengineering is the newest institute of the NIH. With this degree of attention, it may be surprising that there is as yet no universally accepted definition of molecular imaging. Numerous diverse definitions, some quite complex, have been proposed. With some exceptions, they all refer to imaging in the living animal of function at the cellular or molecular level. Thus molecular imaging may be defined as the observation of biological function at the molecular level in health and disease through some process involving non-invasive imaging of the living mammals. J. Cell. Biochem. Suppl. 39: 18–24, 2002.

Key words: molecular nuclear medicine; imaging; PET; SPECT

The brief observations presented herein are intended to show that as defined, nuclear medicine techniques have been in use for molecular imaging for many years and, while there are obvious trade-offs in performance characteristics among all modalities capable of molecular imaging, nuclear medicine should continue to enjoy a position of prominence resulting from the property of extreme detection efficiency. Additionally, while positron emission tomographic (PET) techniques are often considered the preferred approach to molecular imaging in nuclear medicine today, single photon emission tomography (SPECT) offers advantages that not only guarantee its continued use but that may ultimately elevate SPECT to the preferred approach overall. Finally, to the extent that nuclear medicine applied to molecular imaging may achieve prominence in the imaging of gene expression, then both reporter gene imaging and antisense imaging will be important applications. The ability to image the degree, distribution, and persistence of gene expression will unquestionably be useful in connection with gene therapy but being able to image any tissue with a unique genetic expression, as is the promise of antisense imaging, would be revolutionary.

MOLECULAR IMAGING

Several modalities, including autoradiography and electron microscopy, can image at the cellular level but are of no value in the living mammals. Conversely, modalities such as PET, SPECT, computerized X-ray tomography (CT), and magnetic resonance imaging (MRI) are of no value at the cellular level but several of these are of much more interest to researchers concerned with external noninvasive imaging of molecular events in vivo (i.e., molecular imaging). Each is tomographic and therefore, capable of providing 3D images from computer analysis of measurements made around the subject. PET, SPECT, CT, and MRI should

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not be viewed as competing but rather as complementary modalities, each with their own strengths. Many recent studies have shown the advantages of dual-modelity imaging.

CT and MRI are morphological technologies capable of superb spatial resolution. While CT depends upon tissue density (i.e., selective absorption of X-rays), MRI depends upon differences in the chemical environment (i.e., magnetic relaxation) surround water protons and several other nuclei in tissues. However, CT can be described only broadly as a functional imaging technique and only as it applies to gross functions such as blood flow and tissue permeability and then only through the use of heavy metal, high density, contrast agents. Until fairly recently, the same would have to be said of MRI even with the use of paramagnetic contrast agents such as gadolinium-DTPA. But it is now possible to image the distribution of ^{1}H in metabolites as well as ^{31}P in biological molecules such as ATP (i.e., functional MRI or fMRI) and, in limited cases, with tracers (i.e., labeled exogenous molecules) using these and other paramagnetic nuclei [Ross and Michaelis, 1994]. The limitations are poor sensitivity. In the case of ^{1}H, the poor sensitivity is due in part to the large water signal obscuring that of metabolites. In the case of all other nuclei, it is the low concentrations and low signal strengths which explain the poor sensitivity. Concentrations on the order of μM to mM are required for a suitable ^{1}H signal [Brunetti et al., 1996; Luker and Pwinica-Worms, 2001] and at least a log higher concentration is needed in the case of ^{31}P [Ter-Pogossian, 1985]. Thus functional imaging by both CT and MRI are possible only through the use of large concentrations of tracers; concentrations often large enough to provide toxicity and/or to interfere with the function being measured (for example, by saturating low density sites). In addition, the available numbers of such tracers are limited at present. Both CT and MRI do not involve administered ionizing radiation while MRI does not involve ionizing radiation at all. To the extent that low levels of such exposure may be harmful, MRI and possibly CT have this advantage over nuclear medicine techniques. They also tend to be simpler to perform especially if contrast agents or tracer are not required.

An additional technique gaining in prominence is optical imaging (OI). OI can provide high resolution for surface imaging of skin cancers, exposed tissues, and tissues accessible by endoscopy. However, OI is at present limited in the living animal to millimeter-scale resolution due to light scatter and attenuation in deep tissues. Depending upon wavelength and tissue type, the signal can decrease in intensity by a factor of 5–10 per centimeter [Contag et al., 1995]. By being limited to no more than several centimeters of tissue penetration, molecular OI imaging is currently largely restricted to planar imaging of small animals. However, deep tissue approaches and tomographic techniques are currently under development [Ntziachristos and Chance, 2002].

By the nature of the physics involved, nuclear medicine imaging technologies cannot match the spatial resolutions of anatomical CT and MRI (although they can approach that of fMRI [Muller-Gartner, 1998]). However, depending upon the application, this deficiency can be more than compensated for by the extraordinary sensitivities of SPECT and especially PET that may exceed ten logs with respect to MRI [Ter-Pogossian, 1985]. The higher sensitivities make possible in vivo imaging by both SPECT and PET of tracers at physiological concentrations not possible at present with any other technology.

PET is usually considered the leading technology in molecular imaging and superior to SPECT for molecular nuclear medicine imaging for a variety of reasons. The spatial resolution of state-of-the-art PET clinical cameras is about 3–5 mm compared to 6–7 mm for clinical SPECT cameras [Marek and Seibyl, 2000]. In addition, the physiologically important elements carbon, oxygen, and nitrogen have no suitable SPECT radionuclides while each has one suitable positron-emitting radionuclide (albeit with short 20 min, 2 min, and 10 min half lives for ^{11}C, ^{15}O, and ^{13}N, respectively). PET cameras do not need collimation and, as a result, have higher detection sensitivities than SPECT. Because of the need for collimation, SPECT clinical cameras also have lower temporal resolution than PET cameras of about 5–10 min versus 30–50 sec [Muller-Gartner, 1998]. Furthermore, by the nature of the positron decay, absolute activity determinations are routine with PET. These advantages do come at a price however. The short half lives of positron emitters requires an in-house cyclotron for production and greatly complicates the labeling procedures for tracers. Because of its longer

110 min half life, ^{18}F is often used as an alternative PET radiolabel. For example, in the case of ^{18}F radiolabeled glucose (i.e., of ^{18}F-FDG), use of this label has permitted central commercial production and distribution of this labeled glucose. However, its use does not materially simplify the labeling procedure and the result in any case is a radionuclidically substituted biomolecule that may not behave identically to the native biomolecule. Several radionuclide generator products are positron emitters and, while their use can solve the distribution difficulties, like ^{18}F they are in all cases isotopes of non-physiological elements. Finally, PET cameras tend to be more expensive than SPECT cameras. By contrast, the number of suitable single photon emitting radionuclides is fairly large and includes ^{99m}Tc and iodine-123 (^{123}I), both of which are inexpensive, readily available and with almost ideal properties for SPECT imaging. Although iodine (as used here) and technetium are also non-physiological elements, in the past few years methods of attaching these radionuclide to a large variety of biomolecules have been developed often with no detectable influence on biological properties [Wilbur, 1992; Liu and Scott Edwards, 1999]. For ^{99m}Tc in particular, the radiolabeling procedure can also be simple in the extreme. Generally a chelating agent is first covalently attached to the biological molecule and, following purification, the product can be stored until needed at which time the ^{99m}Tc is simply added. Proteins, peptides, DNAs, receptors binding agents, etc. are being radiolabeled in this manner. One particularly important additional advantage of SPECT is the ability to perform multiple labeling studies using two or more radionuclides as tracers simultaneously. The simultaneous use of multiple PET radionuclides is either not convenient or simply not possible since all PET radionuclides decay with the same photon energy.

While PET procedures currently dominate molecular nuclear medicine imaging, SPECT will play in increasingly important role as new ^{99m}Tc and ^{123}I agents are developed. Until recently, the development of new SPECT agents for molecular imaging has been hampered by the absence of a commercial small animal SPECT camera. The development of new radiolabeled agents for molecular imaging will benefit from and will often require the ability to image a single animal multiple times to obtain reliable biodistribution results over time. Just as the development of PET agents has benefited from the availability of the microPET and other small animal PET imagers, the development of SPECT agents will require similar small animal imagers [Acton et al., 2002]. Fortunately, microSPECT cameras are now commercially available with intrinsic resolution of less than 1 mm and, as such, superior to that of small animal PET camera. Furthermore, one commercial small animal camera can generate CT images as well as SPECT images. Coregistered images are acquired almost simultaneously without removing the animal from the imaging system [Patt B., Gamma Medica, personal communication, 2002], something that is not yet possible with commercial PET small animal cameras. With cameras such as this soon to be widely available, SPECT will inevitably play an increasingly important role in molecular nuclear medicine imaging.

MOLECULAR NUCLEAR MEDICINE

There exists a general perception that molecular imaging is a new field, yet molecular nuclear medicine imaging has been in progress for decades, beginning about 50 years ago with the first use of radiolabeled iodide for thyroid imaging. Furthermore, the majority of nuclear medicine imaging procedures in use today has at least a component of molecular imaging. The following table (Table I) was prepared to illustrate this point as it applies to the heart, perhaps the most significant organ with respect to nuclear medicine. The table was prepared as a selected list of the nuclear medicine imaging procedures of the heart currently in clinical use and, in some cases, in late-stage development [Schwaiger and Melin, 1999]. The list is divided into PET and SPECT agent and classified according to function (i.e., imaging target).

Clearly, the imaging of myocardial perfusion heads any list of heart imaging studies in number. However, perfusion imaging relies primarily on passive diffusion and, as such, cannot qualify as molecular imaging. By contrast, measuring the extent and distribution of cell receptors is unquestionably molecular imaging by definition. Although the list of adrenergic and cholonergtic PET receptor imaging radiopharmaceuticals in use in the heart is now quite large, only ^{18}F-fluorodopamine and ^{11}C-epinephrine have been listed here as popular exam-

TABLE I. SPECT and PET Myocardial Imaging Radiopharmaceuticals Classified by Target

Imaging target	SPECT radiopharmaceuticals	PET radiopharmaceuticals
Perfusion	^{99m}Tc-MIBI, tetrofosmin, etc.	^{15}O-water, ^{82}Rb, ^{13}N-ammonia
Receptors	^{123}I-MIBG, ^{99m}Tc-RGD	^{18}F-dopamine, ^{11}C-epinephrine
Metabolism	^{123}I-IPPA, -BMIPP, -DMIPP	^{18}F-FDG, ^{11}C-acetate, ^{11}C-palmitate
Necrosis	^{99m}Tc-pyrophosphate, ^{111}In-antimyosin	None
Hypoxia	^{99m}Tc-HL-91	^{18}F-misonidizole, etc.
Apoptosis	^{99m}Tc-annexin	None

ples. The list of SPECT radiopharmaceuticals for receptor imaging is comparatively limited. Metaiodobenzylguanidine (^{123}I-MIBG) is not strictly a receptor binding agent but since it behaves in vivo like norepinephrine, it accumulates and is stored in presynaptic sympathetic nerve endings. This radiopharmaceutical may therefore be used to image myocardial innervation in cardiac neuropathies, such as that resulting from heart transplant, ventricular tachycardia, and fibrillation. It may also be useful to image the loss of neurons due to ischemia in coronary artery disease and reinnervation with pharmacologic interventions. Also listed is ^{99m}Tc-RGD (i.e., radiolabeled argenine-glycine-asparagine-containing peptides) representing an entirely different class of receptor imaging radiopharmaceutical. A series of proteins, each with this particular tripeptide, display high affinity for the glycoprotein IIb/IIIa receptor on activated platelets. These tripeptides may then be useful eventually for clot imaging. Since angina and myocardial infarction can be initiated by platelet deposition, an effective method of imaging clot formation in arteries would be welcomed.

The principal use of imaging metabolic function is to distinguish viable ischemic myocardium by the perfusion/metabolism mismatch since ischemic myocardia utilized glucose to a degree greater than expected for its reduced perfusion. The imaging of glucose utilization is being accomplished quite successfully with ^{18}F-FDG and PET. Unfortunately, there is not as yet a successful labeled glucose for SPECT imaging. In addition, ^{11}C-acetate is listed as a marker of oxidative metabolism since clearance of this agent reflects myocardial oxygen consumption with uniform washout indicated normal homogenous oxidative metabolism.

Fatty acid metabolism can also be imaged to provide another metabolic measure of tissue viability since fatty acid uptake and utilization is markedly reduced in ischemia in favor of glucose metabolism. One PET agent is ^{11}C-palmitate which behaves identically to natural fatty acids in uptake and metabolism in the myocardium. Fatty acids may also be labeled with ^{123}I and used for the same purpose but with SPECT. One such agent is labeled iodophenypentadecanoic acid (i.e., ^{123}I-IPPA). This is a straight chain fatty acid in contrast to beta-methyl (BMIPP) and dimethyl (DMIPP) that are branched chains. While extraction by the myocardium of the branched chain fatty acids is uninhibited by the methyl group, clearance is delayed due to metabolic trapping. This can facilitate imaging.

For two of the last three targets, there are as yet no PET radiopharmaceuticals. Necrosis can be imaged with ^{99m}Tc-pyrophosphate since this agent binds to calcium in the hydroxyapetite crystals deposited in necrotic tissue. The antimyosin antibody (^{111}In-antimyosin) targets myosin exposed to the circulation due to the breakdown of cell membranes in necrotic tissue. Even though there are PET agents under development that could conceivably image necrosis, they do not target a specific molecule.

An improved imaging agent of myocardial hypoxia would also be welcomed since current approaches to measuring decreased intracellular oxygen tension are indirect and usually provide a cold spot image. The nitromidazols undergo different metabolism in hypoxic versus normoxic cells. Several ^{18}F labeled nitromidazoles have been developed for PET imaging and so have several radioiodinated versions for SPECT studies. Because it is labeled with ^{99m}Tc, HL-91 may be a welcome alternative for SPECT studies. This agent is still under development by Nycomed Amersham and apparently is not an nitromidazol. Another ^{99m}Tc-labeled hypoxic agent currently under development is BRU 59-21 by Bracco Research.

Although apoptosis imaging is currently under consideration primarily in connection with cancer imaging, apoptosis plays an important

role in several cardiovascular diseases. The expression of phosphatidylserine on the cell surface is one useful marker of apoptosis and has been exploited in the development of a radiolabeled annexin V protein. This endogenous protein has a high affinity for phosphatidylserine and has been radiolabeled with both ^{123}I and, more recently, with ^{99m}Tc. The utility of this radiopharmaceutical in heart transplants has been demonstrated in mice showing increased accumulation in proportion to the extent of rejection. There is as yet no comparable PET agent.

Gene Expression Imaging

The field of molecular imaging owes its existence to the revolution in molecular medicine and drug discovery. The completion of the first draft of the human genome and the subsequent developments in proteomics are providing a completely new spectrum of targets for molecular imaging. Among these targets, the first to receive attention is genetic expression. In fact, the term "molecular genetic imaging" has been proposed as a subfield of molecular imaging [Blasberg and Tjuvajev, 2002]. However, it is appropriate to point out that just as molecular imaging been in practice for many years, so has imaging of gene expression. Since proteins are the end product of gene expression, clearly gene expression is being imaged whenever the distribution of receptors and other cellular proteins are imaged. Nevertheless, the conventional imaging of proteins such as these is clearly not among the interests of those intent on molecular imaging of gene expression. Rather the interest is directed more at cellular processes closer to transcription rather than translation. Thus, by various means, reporter gene expression is being imaged primarily with a view to ultimately using the approach in connection with gene therapy [Blasberg and Tjuvajev, 2002] and possibly stem cell therapy.

Gene therapy has shown slow but steady progress thus far over its short decade-long history [French-Anderson, 2002]. Along with one particularly unfortunate failure (the death of a healthy volunteer), there lately has been one spectacular success (the successful treatment of SCID in four children). There are now reported to be at least 2,000 laboratories around the world engaged in gene therapy research with more than 600 clinical trials on record, mostly concerned with the treatment of cancers [Roberts, 2002]. One universal limitation to these on-going studies is the inability at present to establish by any non-invasive method whether transfection of solid target tissue with the vector carrying the therapeutic gene was successful and whether the induced gene expression is persistent. To provide a solution, reporter gene approaches are under investigation in which, in its simplest form, the therapy gene will be transfected together with a second gene (i.e., the reporter gene) in such a manner that both are controlled by the same promoter and therefore, expressed together. Furthermore, the reporter gene will be designed such that its expression may be monitored by external imaging in some fashion. For example, the Herpes simplex virus type 1 thymidine kinase gene (i.e., HSV1-tk) has been widely used as a reporter gene in small animals in studies of proof-of-principle [Wiebe and Knaus, 2001]. The enzymatic protein resulting from gene expression in this case (i.e., HSV1-TK) phosphorates and traps intracellularly nucleosides such as FIAU (a uracil derivitive) or PCV (a guanosine derivitive). When these nucleoside derivatives are radiolabeled, the radiolabel may also be trapped [Ray et al., 2001]. Various mutant forms of these and other reporter genes are under investigation as are other nucleoside derivitives [Tjuvajev, 2002]. Alternatively, radiolabeled agents have also been used to target extracellular receptors (thereby avoiding the need to cross the cell membrane) rather than enzymes present as a result of reporter gene expression. For example, the gene coding for the human somatostatin type 2 receptor (i.e., hSSTr2) has been used as a reporter with FDA-approved and commercially available radiolabeled somatostatin-binding peptides as tracers [Zinn et al., 2002]. Another attractive example may be the sodium iodide symporter. The successful use of the symporter as a reporter gene would permit ^{123}I-radioiodide and ^{99m}Tc-pertechnetate, two of the most common radiopharamaceuticals in clinical use to be used as probes [Chung, 2002].

These and other molecular imaging investigations of reporter gene expression have generally employed radioactivity labels and nuclear medicine imaging, both PET and SPECT, and this is likely to be the case for the foreseeable future. Reporter gene imaging by fMRI has been achieved but is limited by the restricted sensitivity of this modelity such that wide-spread

utility of fMRI for this application must await the development of method for amplifying the signal strength by the deposition in expressed cells of high concentrations of supraparamagnetic metals [Weissleder and Mahmood, 2001]. Also limited in utility for gene expression imaging are OI methods. Reporter gene imaging has also been successfully accomplished with OI under optimal conditions in small animals using near infrared frequencies. Nevertheless, because of light scatter and absorption, it is difficult to envision anything but a limited role for this modelity in human patients [Ray et al., 2001].

A second, but very distinct, approach to imaging gene expression targets messenger RNA (mRNA) rather than proteins since mRNAs are also products of gene expression (i.e., antisense imaging). There is a clear advantage to imaging gene expression in this fashion. That the genetic profiles of many, if not all, healthy and diseased tissues show unique profiles of mRNA expression is now being increasingly demonstrated through the use of cDNA microchips (it remains to be seen whether mRNA profiles are more characteristic than protein profiles). Were it possible to target individual mRNAs in the living animal efficiently and selectively, it may then be possible to successfully image in vivo the distribution of tissues expressing a particular mRNA. Thus any tissue or cell type with an identifiable unique genetic profile would become a candidate for imaging by targeting its unique mRNA expression. For example, there would then be no need for reporter genes since the genetic expression of the therapeutic gene itself could be imaged. It is also possible that the identical or very similar constructs of single chain DNAs or their analogues with base sequences complementary to that of a target mRNA (i.e., antisense DNA) may be universally effective for antisense imaging, differing only in base sequence to accommodate different mRNA targets.

The demonstration that antisense DNAs can interfer with the translation of the mRNAs in connection with antisense chemotherapy [Jacobs, 2002] has stimulated those who wish to use radiolabeled antisense DNA in connection with antisense imaging [Hnatowich, 1999]. In this case, the objective of mRNA targeting is not to interfere with translation but to use targeting as a means of retaining radioactivity in the expressed tissues. While successful treatment by antisense chemotherapy has been demonstrated clinically [Opalinska and Gewirtiz, 2002], proof-of-principle of antisense imaging remains elusive. Although many difficulties remain, many have also been resolved. Antisense DNAs and their analogues have been synthesized that survive nuclease attack, improved methods are now available for identifying base sequences that effectively target particular mRNA, new methods of radiolabeling have been developed and carriers to improve cellular accumulations are under investigation. One major hurdle remaining is the limited counting rates by antisense imaging that may be expected based on limited mRNA targets per cell. The concern is that even the high sensitivity of nuclear imaging may be insufficient to provide a suitable image under these conditions. Fortunately, recent studies suggest that the counting rates achievable may be significantly higher than that predicted on the basis of steady-state mRNA levels [Zhang et al., 2001]. It may be that the more important remaining difficulty is now the poor selectivity as the radiolabeled antisense DNAs are retained in non target tissues. Many more studies are required to evaluate the numerous approaches to improve the status of antisense imaging, including different DNA chemical forms, different methods of radiolabeling, different mRNA targets, different transmembrane carriers, the use of clearing agents, etc. The effort promises to be very worthwhile since antisense imaging of gene expression would appear to be the method of choice were it available.

The recent successful imaging by a reporter gene approach in mice of metastatic prostate cancer following transfection of the primary is particularly encouraging [Adams et al., 2002]. Nevertheless, that hundreds of clinical trials of gene therapy are presently on-going without the benefit of reporter gene imaging argues that its future may be more as a basic research tool of gene therapy rather than a routine imaging procedure. The same could not be said of antisense imaging.

CONCLUSIONS

Molecular imaging is capturing the attention of clinicians and scientists alike as the benefits of the revolution in molecular medicine are applied to in vivo imaging of molecular function. For the first time, we are approaching the

ability to measure in the living animal and by noninvasive techniques, the distribution of enzymes and other proteins, of targeted mRNAs and of expressed genes that until recently were detectable, if at all, only by ex vivo techniques. Among the players, nuclear medicine is dominant in the emergence of molecular imaging because of the extreme sensitivity of which this technique is capable. Until such a time as cellular resolution is needed and achievable by molecular imaging, it may be predicted that molecular nuclear medicine imaging will maintain this position. Within molecular nuclear medicine, the emphasis is currently on PET although this is likely to change as more radiopharmaceuticals are developed labeled with SPECT radionuclides and as small animal cameras designed for SPECT become available. Although OI is also popular as a convenient route to the development in small animals of a clinically useful tool for the measurement of gene expression, the emphasis on nuclear medicine techniques is particularly evident in reporter gene imaging. Reporter gene imaging of gene expression has become the first and best example of what is achievable by modern molecular imaging. Nevertheless, the measurement of gene expression by antisense imaging is arguably a much more powerful technique in principal but, unfortunately, also one apparently more difficult to achieve.

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