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Molecular Imaging: The Application of Small Animal Positron Emission Tomography

Douglas J. Rowland, Jason S. Lewis, and Michael J. Welch*

Radiation Sciences, Mallinckrodt Institute of Radiology, and the Alvin J. Siteman Cancer Center, Washington University School of Medicine, Saint Louis, Missouri

Abstract The extraordinary advances in genomic technologies over the last decade have led to the establishment of new animal models of disease. The use of molecular imaging techniques to examine these models, preferably with non-destructive imaging procedures, such as those offered by positron emission tomography (PET), are especially valuable for the timely advancement of research. With the use of small animal PET imaging it is possible to follow individual subjects of a sample population over an extended time period by using highly specific molecular probes and radiopharmaceuticals. In this *Prospect* small animal PET imaging will be described, specifically focusing on the current technologies, its applications in molecular imaging and the logistics of performing small animal PET. J. Cell. Biochem. Suppl. 39: 110–115, 2002. © 2002 Wiley-Liss, Inc.

Key words: PET; radionuclides; small animal imaging; molecular imaging

The advent of new genomic technologies has led directly to the recent advances in the biomedical sciences and the establishment of transgenic models, generally in mice. Researchers using animal models of disease face unique challenges in the evaluation, analysis, and characterization of these models. It is often advantageous to follow individual subjects of a sample population over an extended time period during which various procedures are performed. Thus, invasive and/or destructive procedures—especially those that require sacrifice of the subject—are prohibitive. Under these circumstances non-destructive, economical imaging procedures, such as those offered by magnetic resonance imaging (MRI), optical imaging, CT scans, and positron emission tomography (PET) are especially valuable for the timely advancement of research. The use of these of imaging modalities modified specifically for use on small animals is particularly attractive [Budinger et al., 1999; Weissleder, 2002].

The non-invasive diagnosis of human diseases has been studied for decades. The advent of PET imaging, an inherent molecular imaging technique, has revolutionized the non-invasive delineation of disease. PET imaging is uniquely able to image the body's basic biochemistry by measuring cell and molecular activity or function. With the use of carefully designed radiolabeled molecular probes PET is able to identify tumor-specific antigens, or genes being expressed. It can also detect signal transductions in neurological systems and diagnose and monitor the development of cancer, for a survey see [Bogdanov et al., 2000; Nichol and Kim, 2001; Pomper, 2001; Chatziloannou, 2002; Lewis et al., 2002; Phelps, 2002]. The design of molecular probes that achieve high target specificity and, therefore, low noise is one of the greatest challenges in PET imaging. PET utilizes radionuclides that decay via positron emission. The positron that is emitted eventually encounters an electron and the two

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*Correspondence to: Michael J. Welch, PhD, Mallinckrodt Institute of Radiology, Washington University School of Medicine, 510 S. Kingshighway Boulevard, Campus Box 8225, Saint Louis, MO 63110.
E-mail: welchm@mir.wustl.edu

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annihilate. Due to the physical characteristics of the annihilation, two 511 keV gamma rays are emitted at 180° with respect to each other. A PET camera detects these gamma rays and after a sufficient number of coincidence events are detected, a three dimensional image can be reconstructed showing where the activity resides within the subject. The use of PET has increased rapidly, and currently 1 million clinical PET studies are being performed in the United States per year (about 10% of the total worldwide diagnostic imaging market). One of the principal reasons for PET growth in the United States is the increasing number of PET applications being approved for payment by Medicare (Centers for Medicare and Medicaid Services, Baltimore, MD) (since July 1, 2001) and private insurers, particularly in the oncology field.

SMALL ANIMAL PET IMAGING

Although perfectly adequate for human study, standard human PET cameras have insufficient resolution for use with rodents. As a consequence, the use of dedicated small animal PET cameras with much higher resolution allow the researcher to carefully evaluate both new tracers and new animal models of disease in an efficient and economic manner. Concurrently researchers continuously refine and reengineer new probe molecules to improve their specificity for molecular imaging of animal models of disease.

Human PET scanners have an inadequate spatial resolution for the imaging of small animals. This inadequacy stems from the nearly three orders of magnitude difference in physical size between rodents and humans. The typical reconstructed image resolution of a full body scan in a human scanner is approximately 10 mm (1 ml in volume). In order to achieve a similar mass-to-image resolution ratio in rodents the reconstructed image resolution should be the order of 1 mm (1 μl in volume). Therefore, with the increased use of small animals—namely rodents—for the study of human disease, much effort has gone into the development of small animal PET cameras to achieve the best possible resolution.

Due to the lack of commercial small animal scanners until recent years, research utilizing these high-resolution devices was limited to research institutions where the human resources and physical infrastructure to design and build such devices were available. These institutions are still advancing the current limits of the technology for the improved imaging of rodents. The engineering behind the recent evolution of small animal PET cameras has led to the development of a number of systems of which two have recently been commercialized. Two recent reviews describe the research and commercial detectors in detail [Chatziloannou, 2002; Lewis et al., 2002].

The commercial detectors are based on two very different concepts. Concorde MicroSystems, Inc. (Knoxville, TN) commercialized the small animal design of a single ring camera built by UCLA Crump Institute (Los Angeles, CA) [Chatziioannou et al., 1999]. This camera uses lutetium oxyorthosilicate (LSO) crystals for the detection of the 511 keV gamma rays. These crystals have the best characteristics of the currently available technology for the most efficient detection of positron annihilation. The microPET design of Concorde MicroSystems uses four rings of 24 detectors each for the R4 rodent model. Each detector consists of an 8×8 array of $2 \times 2 \times 10$ mm LSO crystals. The ring diameter is 22 and 12 cm for the primate and rodent designs, respectively. The axial field of view (FOV) is 8 cm. The absolute sensitivity of these detectors is 2.2 and 2.7% for the primate and rodent cameras, respectively. The measured resolution in the center of the FOV is 1.8 mm but increases to about 2.5 mm at a radial distance of 1 cm [Tai et al., 2001].

The Quad-HIDAC (Oxford Positron Systems, Weston-on-the-Green, UK) is the other commercially available small animal PET camera [Jeavons et al., 1999]. This camera utilizes Multi-Wire Proportional Chamber (MWPC) technology with a High Density Avalanche Chamber (HIDAC). This type of detector uses interleaved lead and insulating sheets with a dense matrix of 0.5-mm holes. Photons from the positron annihilation result in ionization from the interaction of the positron with electrons in the lead plates. Ejected electrons are accelerated in the holes and are collected at an array of anode wires. The pitch of the holes determines the resolution of this type of camera and the interaction probability of the positron with lead determines the sensitivity. The FOV is 17×28 cm and the spatial resolution is reported to be 1.8%.

PROBES

In the design of PET radiopharmaceuticals, important factors to consider include the half-life of the radionuclide, the energy of the radioactive emissions, and the cost and availability of the isotope. For imaging, the half-life of the radionuclide must be long enough to carry out the desired radiochemistry and allow enough time for the radiopharmaceutical to localize in the target tissue following administration. For example, heart or brain perfusion-based radiopharmaceuticals necessitate shorter half-lives, since they reach the target quickly. In order to study the dynamics on a short timescale, a short-lived nuclide is necessary to gather enough statistics for a meaningful image. In contrast tumor-targeted radiopharmaceuticals are often allowed a longer time to reach the target for optimal target tissue to background ratios to be obtained. In selecting which radionuclide to use in PET imaging it is essential to achieve adequate contrast between the target tissue and normal tissue activity levels in a time frame compatible with the physical half-life of the radionuclide.

With small animal PET imaging considerations of the resolution of the isotope also have to be included. ^{18}F is the most common PET isotope and permits the highest resolution for PET due to its low positron energy. ^{64}Cu is another low-energy positron emitter, which gives a similar resolution and yields images comparable to ^{18}F. However, there is increasing interest and need for other PET nuclides which have less than perfect decay characteristics for imaging (e.g., ^{13}N, ^{15}O, ^{60}Cu, ^{66}Ga, ^{76}Br, ^{86}Y, ^{94m}Tc, ^{124}I). This raises several questions about the ability of the small animal PET tomographs to perform high quality imaging with these less than perfect radionulclides [Laforest et al., 2002]. In this respect, the detector size is not necessarily the limiting factor on achieving high resolution and contrast. These isotopes have large positron energies that decrease the image resolution. Some also have a high percentage of prompt gamma rays that fall within the typical energy window for data acquisition. This increases the amount of random coincidence events and thus increases the background; therefore, leading to decreased image contrast. Image resolution and contrast could be improved for these isotopes by refining the machine hardware, the acceptance energy windows, and reconstruction algorithms [Laforest et al., 2002].

APPLICATIONS

In biological systems, the over-expression of cell surface or nuclear receptors is the premise for receptor-based PET radiopharmaceuticals. Small animal PET technology has been used to monitor and delineate a wide variety of biological processes and diseases such as glucose metabolism in the rat brain [Barrio et al., 2000; Kornblum et al., 2000; Moore et al., 2000] and heart [Lapointe et al., 1999], the dopaminergic system [Sargent et al., 1998; Qi et al., 2000], epilepsy [Kornblum and Cherry, 2001], rheumatoid arthritis [Wipke et al., 2002], cell trafficking [Adonai et al., 2002], and inflammatory eye diseases [Wang et al., 2001]. Advances in many areas of genomics, phage display, and proteomics have led directly to progress in hybridoma technology where genetically engineered fragments of antibodies, with rapid access and high retention in tumorous tissues combined with excellent clearance properties, have made the use of such biomolecules suitable for employment in small animal PET imaging [Wu et al., 2000]. Interest in peptides as agents for the diagnosis of cancer has also led to the development of small animal PET imaging as a screening tool to determine optimal peptide sequences for improving the target tissue uptake of radiolabeled peptide analogues [Li et al., 2002; Uger et al., 2002].

Small animal PET imaging is also able to delineate the uptake of a tracer in tissue as a direct measurement of a biological process. This has led to an important application of small animal PET tomographs in defining the kinetics of an imaging agent as a marker to monitor the effectiveness of conventional chemotherapy regimes. One such example has been presented by [Oyama et al., 2001] where they have extrapolated this basic principle by monitoring the effects of androgen therapy on prostate tumors using microPET. They evaluated early changes in tumor metabolism following androgen ablation therapy and the results indicated that changes in serum testosterone levels influence glucose metabolism in the prostate gland within 24 h of treatment. Lewis et al. have also employed the monitoring of therapeutic efficacy. Small animal PET imaging in conjunction with high resolution MRI imaging

yielded information about the response of animal tumors to therapeutic treatment that would not normally be available with the use of traditional caliper measurements [Lewis et al., 2001, 2002].

The extraordinary advances in molecular biology and the direct application of molecular imaging to this field of science has led to the careful design of radiolabeled molecular probes that are targets for reporter gene expression [Gambhir et al., 1999, 2000b; Shao et al., 2000]. Imaging gene expression involves monitoring the expression of a gene product by a reporter gene, which is linked to the gene of the product. A positron-emitting radiolabeled probe will specifically bind to a protein that is being expressed as a result of a gene being administered to an animal and that gene being differentially expressed in target and non-target tissues. Since the same promoter drives both the gene and the reporter they are expressed simultaneously and consequently the amount of tracer retained by the expressed protein is directly proportional to the amount of protein expressed and hence the level of gene expression. With PET probes this allows the investigator to non-invasively monitor and quantify the levels of genes expressed, the time course of expression, as well as the location of genes in animals. For example, 8-[^{18}F]fluoroganciclovir (FGCV), a substrate for the herpes simplex virus 1 thymidine kinase enzyme (HSV1-tk) has been used to image gene expression in normal mice and in transfected tumor cells [Gambhir et al., 1998, 2000a; Iyer et al., 2001; Yaghoubi et al., 2001].

AUTHOR PERSPECTIVES

As discussed by Chatziloannou [2002], there are also practical considerations to the application of small animal PET to the biological sciences. The cost of a small animal scanner is the preliminary consideration. At a cost of greater than $300,000 per scanner, imaging using this modality becomes prohibitive for many laboratories. Another issue that must be addressed with respect to small animal PET technology is the availability of the required radiopharmaceuticals.

What is the infrastructure needed to operate a small animal PET scanner? If the scanner will be run eight or more hours a day, full-time veterinary technicians will be needed to perform anesthesia, microsurgeries, and injections on the animals. Other personnel may also include at least two people to run the scanner and/or analyze and quantify the image data. This also requires a large training/education component for new staff. One final consideration for the use of positron emitting isotopes in research is the safety component. A team of scientists that have not previously used nuclides of this type needs to be educated in the proper use and precautions necessary to work with positron emitters. This includes appropriate lab practices, i.e., shielding, disposal of waste, decontamination of personnel, and/or laboratories.

Concerning the procurement of radioisotopes, two possible approaches may be taken. The production of radioisotopes on site may be implemented. This requires a great deal of infrastructure such as the purchase of a cyclotron and hiring cyclotron operators and radiochemists to produce the radiopharmaceuticals. Alternatively, a laboratory could purchase certain PET isotopes and/or radiopharmaceuticals to perform the required studies. This option would only be available for nuclides that are being produced for shipment by central facilities and that have half-lives that are compatible with the shipping time. For example, one source of ^{18}F is P.E.T.Net Pharmaceuticals (Knoxville, TN), where a network of 32 sites around the country provides ^{18}F-FDG and ^{18}F to local purchasers. Only its short half-life of 2 h limits its shipment to distant sites. Other non-standard PET radionuclides are available through alternative mechanisms. For example, the Research Resource in Radionuclide Research at Washington University in St. Louis has been providing nuclides, such as ^{64}Cu and ^{76}Br to many institutions around the country [McCarthy et al., 2001].

Although the installation of a small animal PET imaging facility may seem daunting, both academic institutions and industry across the world have been purchasing and using small animal PET tomographs. For example, Concorde Microsystems, has delivered 27 systems (through Oct 2002) of which 60% have gone to academic institutions, 30% to industry, and 11% to government institutions. With the expanding availability of small animal PET, researchers without this imaging modality are often able to contact a neighboring facility with the necessary infrastructure to perform the small animal

PET studies as a collaborative project. This is perhaps the most reasonable solution for many scientists interested in small animal PET for molecular imaging who do not have the resources to set up a complete PET laboratory. These collaborating facilities can act both as an extremely valuable resource in the design and implementation of molecular probes but can also supply alternative modalities such as high resolution MRI and CT.

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REFERENCES

Adonai N, Nguyen KN, Walsh J, Iyer M, Toyokuni T, Phelps ME, McCarthy T, McCarthy DW, Gambhir SS. 2002. Ex vivo cell labeling with ^{64}Cu-pyruvaldehyde-bis(N^4-methylthiosemicarbazone) for imaging cell trafficking in mice with positron-emission tomography. Proc Natl Acad Sci USA 99:3030–3035.

Barrio JR, Toyokuni T, Gambhir SS, Cherry SR, Herschman HR, Phelps ME, Satyamurthy N, Kornblum HI. 2000. In vivo imaging of neuronal activation and plasticity in the rat brain by high resolution positron emission tomography (microPET). Nucl Med Biol 27:157–162.

Bogdanov AA, Jr., Simonova M, Weissleder R. 2000. Engineering membrane proteins for nuclear medicine: Applications for gene therapy and cell tracking. Q J Nucl Med 44:224–235.

Budinger TF, Beneron DA, Koretsky AP. 1999. Imaging transgenic animals. Annu Rev Biomed Eng 1:611–648.

Chatziioannou AF, Cherry SR, Shao Y, Silverman RW, Meadors K, Farquhar TH, Pedarsani M, Phelps ME. 1999. Performance evaluation of microPET: A high-resolution lutetium oxyorthosilicate PET scanner for animal imaging. J Nucl Med 40:1164–1175.

Chatziloannou AF. 2002. Molecular imaging of small animals with dedicated PET tomographs. Eur J Nucl Med 29:98–114.

Gambhir SS, Barrio JR, Wu L, Iyer M, Namavari M, Satyamurthy N, Bauer E, Parrish C, MacLaren DC, Borghei AR, Green LA, Sharfstein S, Berk AJ, Cherry SR, Phelps ME, Herschman HR. 1998. Imaging of adenoviral-directed herpes simplex virus type 1 thymidine kinase reporter gene expression in mice with radiolabeled ganciclovir. J Nucl Med 39:2003–2011.

Gambhir SS, Barrio JR, Phelps ME, Iyer M, Namavari M, Satyamurthy N, Wu L, Green LA, Bauer E, MacLaren DC, Nguyen K, Cherry SR, Herschman HR. 1999. Imaging adenoviral-directed reporter gene expression in living animals with positron emission tomography. Proc Natl Acad Sci USA 96:2333–2338.

Gambhir SS, Bauer E, Black ME, Liang Q, Kokoris MS, Barrio JR, Iyer M, Namavari M, Phelps ME, Herschman HR. 2000a. A mutant herpes simplex virus type 1 thymidine kinase reporter gene shows improved sensitivity for imaging reporter gene expression with positron emission tomography. Proc Natl Acad Sci 97:2785–2790.

Gambhir SS, Herschman HR, Cherry SR, Barrio JR, Satyamurthy N, Toyokuni T, Phelps ME, Larson SM, Balatoni J, Finn R, Sadelain M, Tjuvajev J, Blasberg R. 2000b. Imaging transgene expression with radionuclide imaging technologies. Neoplasia 2:118–138.

Iyer M, Barrio JR, Namavari M, Bauer E, Satyamurthy N, Nguyen K, Tokokuni T, Phelps ME, Herschman HR, Gambhir SS. 2001. 8-[^{18}F]Fluoropenciclovir: An improved reporter probe for imaging HSV1-tk reporter gene expression in vivo using PET. J Nucl Med 42:96–105.

Jeavons AP, Chandler RA, Dettmar CAR. 1999. A 3D HIDAC-PET camera with sub-millimeter resolution for imaging small animals. IEEE Trans Nucl Sci 46:468–473.

Kornblum HI, Cherry SR. 2001. The use of microPET for the development of neural repair therapeutics: Studies in epilepsy and lesion models. J Clin Pharmacol Suppl 41: 55S–63S.

Kornblum HI, Araujo DM, Annala AJ, Tatsukawa KJ, Phelps ME, Cherry SR. 2000. In vivo imaging of neuronal activation and plasticity in the rat brain by high resolution positron emission tomography (microPET). Nat Biotech 18:655–660.

Laforest R, Rowland DJ, Welch MJ. 2002. MicroPET imaging with nonconventional isotopes. IEEE Trans Nucl Sci 49 (in press).

Lapointe D, Bentourkia M, Cadorette J, Rodique S, Ouellet R, Benard F, Van Lier JE, Lecomte R. 1999. High-resolution cardiac PET in rats. J Nucl Med 40:185p.

Lewis JS, Buettner TL, Connett JM, Fujibayashi Y, Welch MJ. 2001. Cu-64-diacetyl-bis(N^4-methylthiosemicarbazone): An agent for radiotherapy. Proc Natl Acad Sci USA 98:1206–1211.

Lewis JS, Achilefu S, Garbow JR, Laforest R, Welch MJ. 2002. Small animal imaging: Current technology and perspectives for oncological imaging. Eur J Cancer 38: 2173–2188.

Li WP, Lewis JS, Kim JY, Bugaj JE, Johnson MA, Erion JL, Anderson CJ. 2002. DOTA-D-Tyr1-octreotate: A somatostatin analog for labeling with metal and halogen radiolnuclides for cancer imaging and therapy. Bioconjug Chem 13:721–728.

McCarthy DW, Bigott HM, Perkins TA, Voller TF, Welch MJ. 2001. A research resource in radionuclide research. J Labelled Cpd Radiopharm 44:S817–S819.

Moore TH, Osteen TL, Chatziioannou TF, Hovda DA, Cherry TR. 2000. Quantitative assessment of longitudinal metabolic changes in vivo after traumatic brain injury in the adult rat using FDG-microPET. J Cereb Blood Flow Metab 20:1492–1501.

Nichol C, Kim EE. 2001. Molecular imaging and gene therapy. J Nucl Med 42:1368–1374.

Oyama N, Jones LA, Sharp TL, Welch MJ. 2001. Androgenic control of glucose and acetate metabolism in rat

prostate and prostate cancer tumor model. J Nucl Med 42:26p.

Phelps ME. 2002. Nuclear medicine, molecular imaging, and molecular medicine. J Nucl Med 43:13N–14N.

Pomper MG. 2001. Molecular imaging: An overview. Acad Radiol 8:1141–1153.

Qi J, Moore A, Annala A, Nguyen K, Leahy R, Cherry SR, Araujo DM. 2000. Deficits in striatal dopamine D(2) receptors and energy metabolism detected by in vivo microPET imaging in a rat model of Huntington's disease. IEEE Trans Med Imaging 19:507–512.

Sargent PA, Bench CJ, Rabiner EA, Osman S, Pike VW, Hume SP, Grasby PM, Lammertsma AA. 1998. Positron emission tomography (PET) methodology for small animals and its application in radiopharmaceutical preclinical investigation. Neuroimage 8:426–440.

Shao Y, Silverman RW, Cherry SR, MacLaren DC. 2000. PET imaging of transgene expression. Med Phys 27: 1535–1543.

Tai YC, Chatziioannou A, Siegel S, Young J, Newport D, Goble RN, Nutt RE, Cherry SR. 2001. Performance evaluation of the microPET-P4: A PET system dedicated to animal imaging. Phys Med Biol 46:1845–1862.

Uger O, Kothari PJ, Finn RD, Zanzonico P, Ruan S, Guenther I, Maecke HR, Larson SM. 2002. Ga-66 labeled somatostatin analogue DOTA-DPhe1-Tyr3-octreotide as a potential agent for positron emission tomography imaging and receptor mediated internal radiotherapy of somatostatin receptor positive tumors. Nucl Med Biol 29: 147–157.

Wang Z, Sharp TL, Bora NS, Sohn J-H, Cruz JM, Shao H, Kaplan HJ, McCarthy TJ. 2001. Approaches toward the in vivo imaging of experimental acute autoimmune uvitis (EAAU). J Labelled Cpd Radiopharm 44:S372–S373.

Weissleder R. 2002. Scaling down imaging: Molecular mapping of cancer in mice. Nat Rev 2:1–8.

Wipke BT, Wang Z, Kim J, McCarthy TJ, Allen PM. 2002. Dynamic visualization of a joint-specific autoimmune response through positron emission tomography. Nat Immunol 3:336–372.

Wu AM, Yazaki PJ, Tsai S, Nguyen K, Anderson AL, McCarthy DW, Welch MJ, Shively JE, Williams LE, Raubitschek AA, Wong JY, Toyokuni T, Phelps ME, Gambhir SS. 2000. High-resolution microPET imaging of carcinoembryonic antigen-positive xenografts by using a copper-64-labeled engineered antibody fragment. Proc Natl Acad Sci USA 97:8495–8500.

Yaghoubi SS, Wu L, Liang Q, Toyokuni T, Barrio JR, Namavari M, Satyamurthy N, Phelps ME, Herschman HR, Gambhir SS. 2001. Direct correlation between positron emission tomographic images of two reporter genes delivered by two distinct adenoviral vectors. Gene Ther 8:1072–1080.