European Journal of Pharmaceutics and Biopharmaceutics 74 (2010) 50–54

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Small-animal PET: A promising, non-invasive tool in pre-clinical research

Uta Schnöckel [a,*], Sven Hermann [a], Lars Stegger [a], Marilyn Law [a], Michael Kuhlmann [b], Otmar Schober [a], Klaus Schäfers [b], Michael Schäfers [b]

[a] Klinik und Poliklinik für Nuklearmedizin, Universitätsklinikum Münster, Münster, Germany
[b] European Institute of Molecular Imaging, Universitätsklinikum Münster, Münster, Germany

ARTICLE INFO

Article history:
Received 15 January 2009
Accepted in revised form 22 May 2009
Available online 29 May 2009

Keywords:
Small-animal PET
Nuclear medicine
Pre-clinical research
Molecular imaging

ABSTRACT

Today, non-invasive imaging techniques are significantly contributing to the understanding of molecular processes *in vivo*. Positron emission tomography (PET) is a scintigraphic medical imaging modality that uses radiolabelled molecules (tracers), provides quantitative tomographic images and allows non-invasive assessment of the biodistribution of radioactive substances *in vivo*. The assessment of pathological glucose metabolism is the clinically best-established application of PET today; however, a multitude of different tracers are available to assess diverse physiological processes. The growing interest in pre-clinical imaging studies, in biological and medical basic research, as well as in pharmaceutical research, has fostered the recent growth in small-animal PET. Small-animal PET can be applied to enable the transfer from molecular findings in vitro to *in vivo* applications in humans, from bench to bed side.

1. Introduction

Molecular biology has promoted the development of novel diagnostics and therapies targeting pathological processes on a molecular level. However, findings *in vitro* cannot necessarily be translated to the *in vivo* situation with its associated molecular interactions. Obviously, human studies would be best to study pathophysiological mechanism *in vivo*, these are often impossible due to ethical concerns [1]. Therefore, small-animal models represent an important bridge between discoveries at the molecular level and implementation of clinically relevant diagnostics or therapeutics.

Studies of small-animal models had previously been based on *ex vivo* tissue sectioning and microscopy. In the case of radionuclide-based assays, tissue gamma counting and autoradiography postmortem are traditional tools in small-animal diagnostics [1]. However, these methods do not allow to study a single animal serially over time and require the assembling of histologic or autoradiographic sections. In addition, in many cases assemblies of multiple experiments from different animals are necessary to get information about a molecular process. In this regard, imaging-based methods such as positron emission tomography (PET) have become important non-invasive techniques for the characterisation of mouse models *in vivo* [2]. PET offers excellent insight into biochemical changes on a molecular level, with high sensitivity [3].

2. History of PET

Ter Pogossian performed the first human brain PET study in 1975 [4]. Thirty years later, PET has become an established tool in routine diagnostic work up in the diagnosis of cancer, inflammation, cardiac disease or neurological disorders [2].

Initially, PET imaging of small animals, especially mice, was limited by the technology. The spatial resolution achievable permitted only imaging of rather large tissue regions in rats [5]. In 1991, Ingvar et al. published the first PET image of a rat brain using a Scandatronix PC-2048-15B clinical PET scanner [6]. The first dedicated small-animal PET scanner RATPET was introduced in 1995 [7]. Since then, the development of small-animal PET has become a promising area for technological innovation. Spatial resolution has approached and even exceeded the limit of 1 mm now [2].

3. Technical background

PET uses radionuclides that undergo a β^+ decay with the emission of a positron. The positron, being the antiparticle of the electron, travels a short distance within the tissue before it annihilates with one of the abundant electrons. Governed by energy and momentum conservation laws, the energy equivalent of the positron and electron mass ($E = m * c^2$) is converted into two 511-keV gamma photons that travel essentially in opposite directions (it can be ignored that more than two photons are created in a fraction of cases). The PET device has to detect this pair of 511-keV photons. The technique depends on simultaneous or coincidental detection of this pair of photons (Fig. 1). There are two major

* Corresponding author. Klinik und Poliklinik für Nuklearmedizin, 48149 Münster, Germany. Tel.: +49 251 83 47853; fax: +49 251 83 47685.
E-mail address: uta.schnoeckel@uni-muenster.de (U. Schnöckel).

0939-6411/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2009.05.012

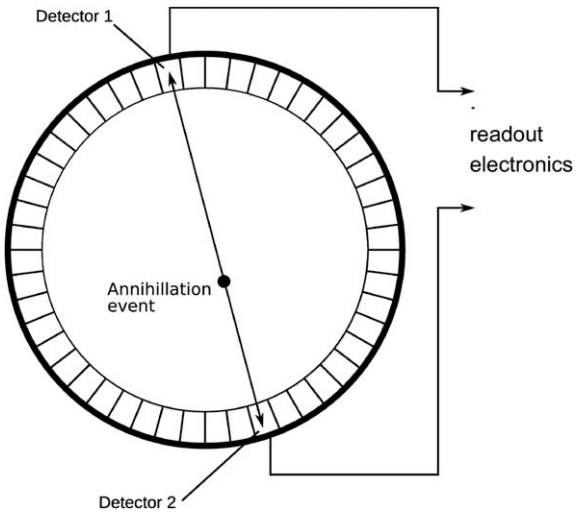

Fig. 1. PET has to detect a pair of 511-keV photons produced from positron annihilation. The technique depends on simultaneous or coincidental detection of this pair of photons.

principles of the technical design of a detector for counting photons secondary to a positron decay. The first one uses photon-sensitive crystals for photon detection, which convert incoming gamma rays into light and further into electrons. This can be measured and amplified with dedicated electronic devices. The second principle is based on a multi-wire gas chamber. The 511-keV photons are first converted into electrons. Afterwards, electrons are multiplied within the gas by help of an electrical field (avalanche effect) and detected by anode wires. This detector principle is used in the quadHIDAC-PET scanner [8] (Oxford Positron Systems Ltd., Weston-on-the-Green, UK), which was used to produce all images within this article.

This scanner currently provides the best resolution, even though crystal-based scanner installations worldwide far outnumber HIDAC scanner installations. The quadHIDAC consists of laminated cathodes containing interleaved lead and insulated sheets, mechanically drilled with a dense matrix of small holes. Holes act like small detectors where an electron, which may originate from photon interaction with the surrounding lead, is trapped, amplified by the avalanche effect in a strong electrical field and detected by anode wires. Sixteen or thirty-two modules are arranged as four detector banks. The quadHIDAC scanner has a field of view (FOV) of 280 mm axially and 170 mm in diameter. The volumetric spatial resolution is with 1.09 mm^3 nearly constant over the whole FOV. The absolute sensitivity in the center of the FOV is 18 cps/kBq (32-module model).

4. Tracer principle

Radioactive tracers are substances labelled with a radioactive atom to allow the measurement of its distribution, e.g. after intravenous injection. Tracers principally consist of two components: firstly a molecule that binds on a specific target and governs the biodistribution of the tracer and secondly a radioactive isotope allowing detection in a gamma camera or a PET scanner. Tracers used in scintigraphic methods are also nominated as radiopharmaceuticals. By detecting the radioactive decay it is possible to assess the distribution as well as the metabolism of a tracer. Radionuclides emit gamma radiation (gamma camera) or positrons (PET).

Some radiopharmaceuticals are chemically identical to the unlabelled original substances. To synthesise these tracers single atoms are replaced by radioactive isotopes without changing the chemical structure of the molecule. Since the organism is not able to differentiate between different isotopes, these tracers are handled by the organism exactly as the original molecule.

However, most of the used radiopharmaceuticals are so-called analogue radiopharmaceuticals that do not exactly correspond to the original molecule but exhibits a similar behaviour. One example of an analogue tracer is C-11 labelled meta-hydroxyephedrin (mHED) that is very similar to noradrenalin and follows the same biochemical route. However, mHED is not metabolised by monoaminooxidase (MAO) as fast as noradrenalin, leaving enough time for PET detection. Another example of a beneficial effect by modifying the structure of a molecule is the clinically established PET tracer F-18-deoxyglucose (FDG) that is – in contrast to glucose – trapped intracellularly (see below).

5. PET tracers

Positron emitters are used for labelling different biochemical molecules in very low mass amounts as PET tracers. The most commonly used positron emitters are O-15, N-13, C-11 and F-18 with physical half-live times between 2.05 min (O-15) and 109.7 min (F-18) [9]. Due to their short half lives, these isotopes must be produced at or near the site of the PET scanner, most often in a cyclotron.

FDG is the most common and clinically established F-18-labelled tracer. It is used for many applications, with the majority of studies performed for tumour imaging, imaging of inflammatory and neuronal pathologies, as well as imaging of cardiovascular diseases. FDG follows the initial biochemical route of glucose. Following injection, FDG is taken up by the cells and phosphorylated. In contrast to glucose, FDG is not a suitable target for the glucose-fructose isomerase so that it is effectively trapped intracellularly for a considerable time (Fig. 2). However, FDG is not a "specific" radiotracer, since all living cells need glucose [10].

In addition to FDG, many other more specific radiopharmaceutical tracers can be used, depending on the clinical question. Some of these examples are described below.

6. Applications in cardiovascular disease

Cardiovascular diseases are the leading cause of death in industrialised countries. A multitude of transgenic, knockout and interventional murine models have been established to mimic human diseases [1]. Phenotypisation of these models with respect to cardiac parameters, such as perfusion and metabolism, have already been demonstrated. Imaging myocardial viability after infarction in a mouse heart using FDG-PET is already possible (Fig. 3). Nevertheless, imaging the heart of a mouse *in vivo* requires a very high spatial resolution due to the small dimension of the structures.

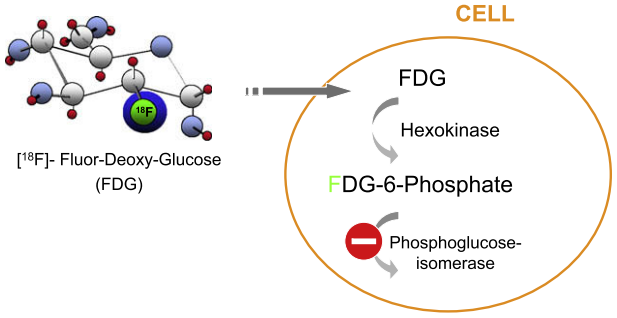

Fig. 2. F-18-fluordesoxyglucose (FDG) is the most common and clinically established PET tracer. After injection, FDG is taken up by the cells and phosphorylated. In contrast to glucose, FDG is not a suitable target for the phosphoglucoisomerase. It is trapped intracellularly for a considerable time.

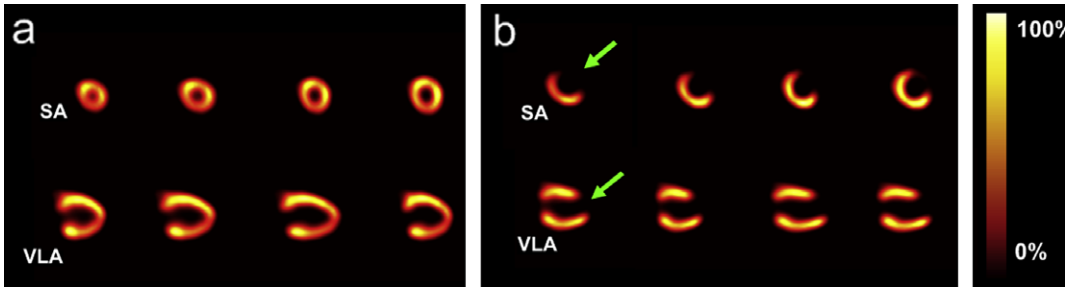

Fig. 3. PET scan of mouse heart after injection of 10 MBq FDG at baseline (a) and 7 d after myocardial infarction (MI) (b). Arrow indicates the infarcted tissue after MI in the anterior apex. Short-axis (SA) slices from apex to base and vertical long-axis (VLA) slices from septum to lateral wall show "human-like" image quality in mice.

In addition, the heart of mice beats at a high rate (400–800 beats/min) and moves with every single beat. Therefore, acquisition of images must often be performed with cardiac gating [1]. A breakthrough in cardiac molecular imaging in mice was the successful electrocardiogram (ECG)-gated PET imaging of the mouse heart in 2005 [11,12]. Small-animal FDG-PET has been validated for reliable and serial investigations of functional cardiac parameters such as ventricular volumes and ejection fraction. F-18-FDG-PET allows for combined molecular and functional imaging of the left ventricle within a single scan, obviating additional e.g. magnetic resonance imaging (MRI) in many cases [3].

In addition to F-18-FDG, many other more specific radiotracers can be used in the diagnosis of cardiovascular diseases. These tracers allow for the measurement of receptor densities (e.g. β-adrenoreceptor density) [13,14]. Other tracers like F-18-labelled annexin V [15] or F-18-labelled caspase inhibitors [16] are promising tracers for apoptosis imaging. Targeting vascular endothelial factors, ischemia induced angiogenesis or plaques are other fields of interest in PET-based cardiovascular imaging.

7. Applications in oncology and inflammation

Experimental tumours in small animals are commonly used as biological models in oncology research. Subcutaneous and orthotopic murine models of different human tumour cell lines are accessible and a huge variety of transgenic/knock-out mouse models have been established [2]. While FDG is widely used for tumour imaging in clinical oncology, it has also been applied to measure glucose metabolism in tumours of small animals. Dedicated small-animal PET systems are able to visualise FDG uptake even within a small tumour. In addition, whole body PET allows identification and monitoring of metastatic spread. Small-animal PET has the major advantage to be non-invasive, permitting serial studies in the same animal.

One example of the potential of PET in inflammatory diseases is the imaging of gastrointestinal graft-versus-host disease (GVHD), which is a common and potentially life-threatening complication after allogenic hematopoietic stem-cell transplantation. Stelljes et al. have shown that FDG-PET is a potent non-invasive technique to assess and monitor intestinal GVHD in mice (Fig. 4) and in humans [17].

New drugs and probes have been radiolabelled to investigate their pharmacokinetics and pharmacodynamics non-invasively *in vivo* using small-animal and clinical PET in oncology. Examples are the F-18-labelled fluconazole, an antimycotic agent, and F-18-fluoropaclitaxel, an F-18-labelled analogue of the cytostatic agent paclitaxel [18,19]. Another example of a radiolabelled drug used to determine the pharmacokinetics and pharmacodynamics of an anticancer prodrug was C-11 labelled temozolomide.

8. Applications in neurology

Imaging the brain in small animals possesses a challenge especially because of the small size of brain structures. Another chal-

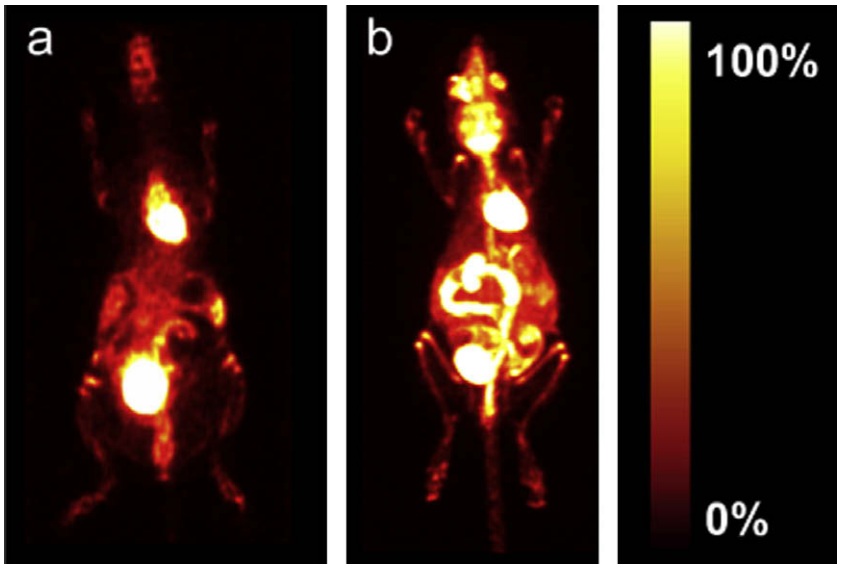

Fig. 4. *In vivo* imaging of a mouse after application of 10 MBq FDG with a quadHidac scanner baseline (a) and 21 days after stem-cell transplantation (b) demonstrated an increased FDG uptake in the colon of the animal after transplantation of graft-versus-host disease.

lenge is the low density of interested targets. Brain imaging, therefore, demands an imaging device with both a high spatial resolution and a high sensitivity at the same time. While radionuclide-based imaging has a very high sensitivity but a limited resolution, conventional imaging methods such as computer tomography or MRI has a high resolution for precise anatomical information, but a low sensitivity for molecular targets. Therefore, it is beneficial to coregister small-animal PET data of the brain with morphological imaging data e.g. with MRI. The coregistered data improve spatial delineation of the area of interest and allow for partial volume correction of the radionuclide data to increase quantification accuracy. Retrospective data coregistration in the brain has been used to a considerable extent.

Using small-animal PET, it is possible to monitor neurotransmitter function *in vivo* over time [21,22]. One example for a neurological animal model is the heterocygote transgenic rat model of Huntington's disease. Araujo et al. described investigations with FDG and fluoroethylspiperone to study the time course of changes in glucose metabolism and dopaminergic function in a rat model of Huntington's disease [23]. Such longitudinal non-invasive studies provide data that could not be obtained in any other way and represent the ideal application of small-animal PET.

9. Acquisition of kinetic data

Because of the excellent resolution of small-animal PET scanners, it is able to acquire time–activity profiles reflecting tracer delivery, binding and washout of very small regions of interest (ROI) [5]. Compared to invasive tools, inter-animal variation is removed, which reduces the number of required animals, benefiting both cost and ethical issues. Especially, when assessing the biodistribution of a new drug dynamic small-animal PET allows for an overall time-depending assessment of the compound.

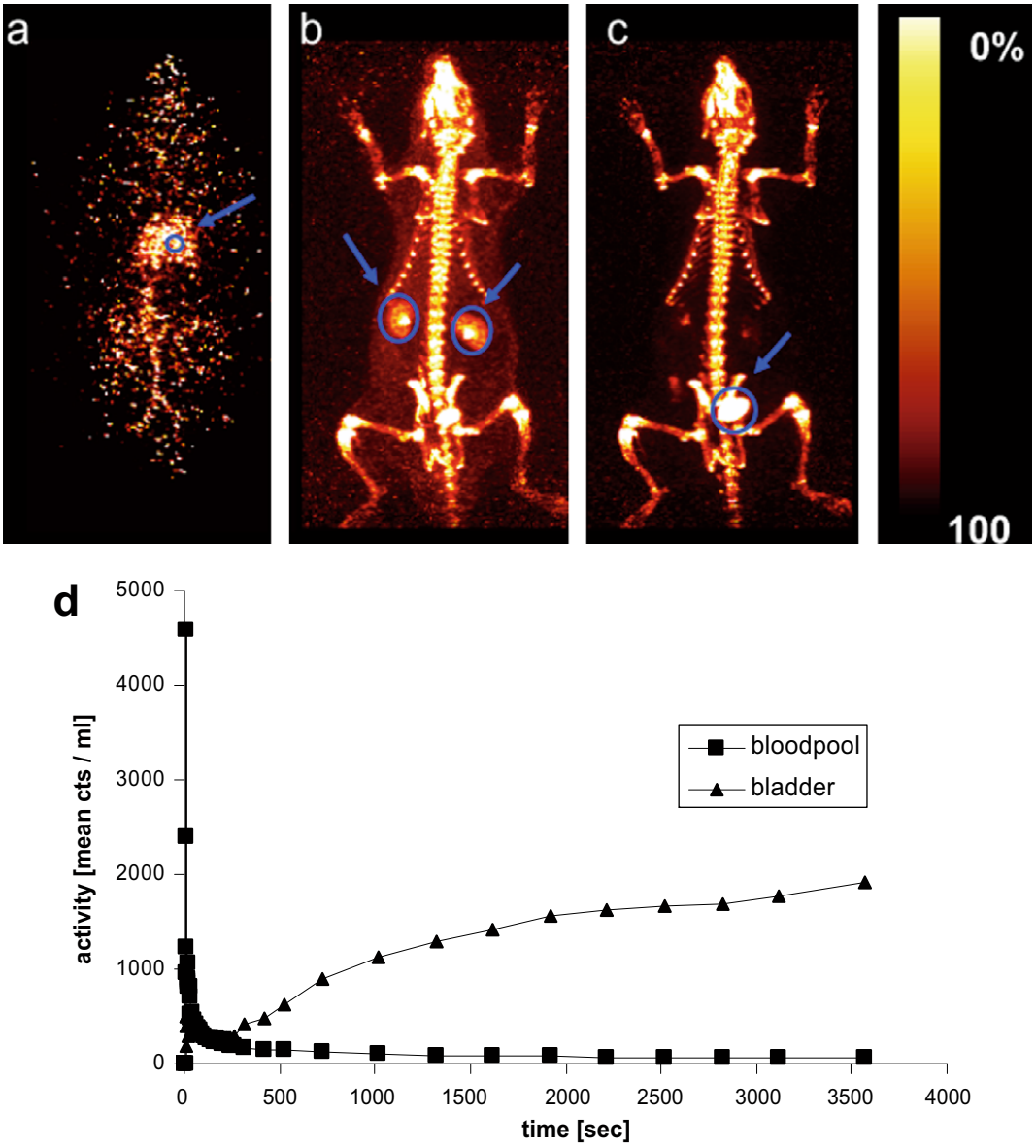

Fig. 5. Representative images of a dynamic whole body acquisition of a control rat after i.v. injection of 15 MBq 18F-fluoride (maximum a posterior projections (MAP)), mid-rat coronal slice 6–8 s p.i. (arrow: heart) (a), 120–130 s p.i. (arrows: kidneys) (b) and 3300–3600 s p.i. (arrow: bladder) (c). Representative time activity curve (d) of the arterial input function and the activity excreted in the urinary bladder created from the volumes of interest, which were assigned over the blood pool of the left ventricle during the perfusion in the first seconds and over the urinary bladder 3600 s p.i.: 54 time frames (1 * 30 s, 15 * 2 s, 10 * 6 s, 12 * 10 s, 3 * 20 s, 3 * 100 s, 10 * 300 s).

In addition, collection of kinetic data enables the calculation of functional parameters such as perfusion or clearance of radiolabelled substances. An example is the non-invasive assessment of renal F-18 clearance as a parameter of renal function in rats, calculated by using the blood pool activity and the total renal excreted activity (Fig. 5) [24].

10. Multimodality PET imaging

PET is well known for its ability to assess molecular pathways non-invasively *in vivo* in both pre-clinical and clinical studies with very high sensitivity. However, PET cannot assess morphology and is, therefore, ideally combined with morphological imaging devices in hybrid systems. Ultrasound offers excellent spatial and temporal resolution, and thus provides information on both organ morphology and function; the recent introduction of tomographic data acquisition facilitates coregistration with PET. CT offers tomographic datasets of morphology with a high spatial but limited temporal resolution. MRI produces images with a very good soft tissue contrast and can additionally depict many functional parameters of cardiac movement and blood flow. Hybrid imaging technologies such as PET/CT or PET/MRI enable the assessment of complementary aspects of diseases, namely morphology, function and molecular pathways, in a one-stop-shop fashion [25]. PET/CT has been available for several years now for both animals and humans that allows for *sequential* PET and CT data acquisition without repositioning. This gives optimal spatial coregistration, but not yet optimal temporal coregistration for processes with fast tracer kinetics. Several prototype PET/MRI scanners have been constructed that allow for PET and MRI data acquisition *at the same time* for optimal spatial *and* temporal coregistration, making PET/MRI a very promising research and clinical tool [20].

11. Conclusions

Due to the growth in interest in small-animal imaging of physiological processes on the molecular level, dedicated small-animal PET cameras have been developed over the past decade. Development has been brought forward by demand for drug development and the need for tools to translate understanding of fundamental molecular processes at the cellular level to clinically relevant applications. A multitude of established and novel radiotracers are available in a fast growing number. Hybrid PET/CT and PET/MRI imaging for concurrent molecular, morphological and functional imaging is an important current development. All in all, small-animal PET plays an important role in bridging the gap between basic, pre-clinical and clinical research and clinical application [26].

Acknowledgments

This work was supported in part by the SFB 656 "Molecular Cardiovascular Imaging", Münster and the Interdisciplinary Center of Clinical Research Münster, core unit "Small Animal PET" (SmAP).

References

[1] B.L. Franc, P.D. Acton, C. Mari, B.H. Hasegawa, Small-animal SPECT and SPECT/CT: important tools for preclinical investigation, J. Nucl. Med. 49 (10) (2008) 1651–1663.
[2] B. Riemann, K.P. Schäfers, O. Schober, M. Schäfers, Small animal PET in preclinical studies: opportunities and challenges, Q. J. Nucl. Med. Mol. Imaging 52 (3) (2008) 215–221.
[3] L. Stegger, E. Heijman, K.P. Schäfers, K. Nicolay, M.A. Schäfers, G.J. Strijkers, Quantification of left ventricular volumes and ejection fraction in mice using PET, compared with MRI, J. Nucl. Med. (2008) 17 (Epub ahead of print).
[4] M.M. Ter Pogossian, M.E. Phelps, E.J. Hoffmann, N.A. Mullani, A positron-emission transaxial tomograph for nuclear imaging (PETT), Radiology 114 (1975) 89–98.
[5] R. Myers, S. Hume, Small animal PET, Eur. Neuropsychopharmacol. 12 (6) (2002) 545–555.
[6] M. Ingvar, L. Eriksson, G.A. Rogers, S. Stone-Elanders, L. Widen, Rapid feasibility studies of tracers for positron emission tomography: high-resolution PET in small animals with kinetic analysis, J. Cereb. Blood Flow Metab. 11 (1991) 926–931.
[7] P.M. Bloomfield, S. Rajeswara, T.J. Spiks, S.P. Hume, R. Myers, S. Ashworth, et al., The design and physical characteristics of a small animal positron emission tomograph, Phys. Med. Biol. 40 (1995) 1105–1126.
[8] K.P. Schäfers, Imaging small animals with positron emission tomography, Nuklearmedizin 42 (3) (2003) 86–89 (Erratum in: Nuklearmedizin 42(6) (2003) 254).
[9] S. Ziegler, Physical principles, dedication/coincidence PET, in: P. Oehr, H.-J. Biersack, R.E. Coleman (Eds.), PET and PET/CT in Onology, Springer-Verlag, Berlin Heidelberg, 2004, pp. 3–4.
[10] E.K. Pauwels, M.J. Ribeiro, J.H. Stoot, V.R. McCready, M. Bourguignon, B. Mazière, FDG accumulation and tumor biology, Nucl. Med. Biol. 25 (4) (1998) 317–322.
[11] K.P. Schäfers, L. Stegger, C. Barnard, M. Kriens, S. Hermann, O. Schober, M. Schäfers, ECG-triggered high-resolution positron emission tomography: a breakthrough in cardiac molecular imaging of mice, Eur. J. Nucl. Med. Mol. Imaging 32 (3) (2005) 383.
[12] Y. Yang, S. Rendig, S. Siegel, D.F. Newport, S.R. Cherry, Cardiac PET imaging in mice with simultaneous cardiac and respiratory gating, Phys. Med. Biol. 50 (13) (2005) 979–989.
[13] M.P. Law, S. Wagner, K. Kopka, V.W. Pike, O. Schober, M. Schäfers, Are [O-methyl-(11)C] derivatives of ICI 89,406 beta(1)-adrenoceptor selective radioligands suitable for PET?, Eur J. Nucl. Med. Mol. Imaging 35 (1) (2008) 174–185.
[14] K. Kopka, M.P. Law, H.J. Breyholz, A. Faust, C. Höltke, B. Riemann, O. Schober, M. Schäfers, S. Wagner, Non-invasive molecular imaging of beta-adrenoceptors in vivo: perspectives for PET-radioligands, Curr. Med. Chem. 12 (18) (2005) 2057–2074.
[15] Y. Murakami, H. Takamatsu, J. Taki, M. Tatsumi, A. Noda, R. Ichise, J.F. Tait, S. Nishimura, 18F-labelled annexin V: a PET tracer for apoptosis imaging, Eur. J. Nucl. Med. Mol. Imaging 31 (4) (2004) 469–474.
[16] A. Faust, S. Wagner, M.P. Law, S. Hermann, U. Schnöckel, P. Keul, O. Schober, M. Schäfers, B. Levkau, K. Kopka, The nonpeptidyl caspase binding radioligand(S)-1-(4-(2-[18F]Fluoroethoxy)-benzyl)-5-[1-(2 methoxymethylpyrrolidinyl) sulfonyl]isatin ([18F]CbR) as potential positron emission tomography-compatible apoptosis imaging agent, Q. J. Nucl. Med. Mol. Imaging 51 (1) (2007) 67–73.
[17] M. Stelljes, S. Hermann, J. Albring, G. Köhler, M. Löffler, C. Franzius, C. Poremba, V. Schlösser, S. Volkmann, C. Opitz, C. Bremer, T. Kucharzik, G. Silling, O. Schober, W.E. Berdel, M. Schäfers, J. Kienast, Clinical molecular imaging in intestinal graft-versus-host disease: mapping of disease activity, prediction, and monitoring of treatment efficiency by positron emission tomography, Blood 111 (5) (2008) 2909–2918.
[18] A.J. Fischman, N.M. Alpert, E. Livni, S. Ray, I. Sinclair, D.R. Elmaleh, S. Weiss, J.A. Correia, D. Webb, R. Liss, et al., J. Pharmacol. Exp. Ther. 259 (3) (1991) 1351–1359.
[19] K.A. Kurdziel, J.D. Kalen, J.I. Hirsch, J.D. Wilson, R. Agarwal, D. Barrett, H.D. Bear, J.F. McCumiskey, Imaging multidrug resistance with 4-[18F]fluoropaclitaxel, Nucl. Med. Biol. 34 (7) (2007) 823–831.
[20] B.J. Pichler, H.F. Wehrl, A. Kolb, M.S. Judenhofer, Positron emission tomography/magnetic resonance imaging: the next generation of multimodality imaging?, Semin Nucl. Med. 38 (3) (2008) 199–208.
[21] E.M. Torres, R.A. Fricker, S.P. Hume, R. Myers, J. Opacka-Juffry, S. Ashworth, D.J. Brooks, S.B. Dunnett, Assessment of striatal graft viability in the rat in vivo using a small diameter PET scanner, Neuroreport 6 (15) (1995) 2017–2021. Oct 23.
[22] R.A. Fricker, E.M. Torres, S.P. Hume, R. Myers, J. Opacka-Juffrey, S. Ashworth, D.J. Brooks, S.B. Dunnett, The effects of donor stage on the survival and function of embryonic striatal grafts in the adult rat brain. II. Correlation between positron emission tomography and reaching behaviour, Neuroscience 79 (3) (1997) 711–721.
[23] D.M. Araujo, S.R. Cherry, K.J. Tatsukawa, T. Toyokuni, H.I. Kornblum, Deficits in striatal dopamine D(2) receptors and energy metabolism detected by in vivo microPET imaging in a rat model of Huntington's disease, Exp. Neurol. 166 (2) (2000) 287–297.
[24] U. Schnöckel, S. Reuter, L. Stegger, E. Schlatter, K.P. Schäfers, S. Hermann, O. Schober, G. Gabriëls, M. Schäfers, Dynamic (18)F-fluoride small animal PET to noninvasively assess renal function in rats, Eur. J. Nucl. Med. Mol. Imaging 35 (12) (2008) 2267–2274.
[25] N. Lang, S. Hermann, S. Hold, M. Mienkina, L. Stegger, J. Stypmann, O. Schober, M. Schäfers, K.P. Schäfers, G. Schmitz, Hybrid 3D Sono/PET in a mouse, Eur. J. Nucl. Med. Mol. Imaging 34 (10) (2007) 1706–1707.
[26] M.E. Phelps, PET: the merging of biology and imaging into molecular imaging, J. Nucl. Med. 41 (4) (2000) 661–681.