Applications of Small Animal PET

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Abstract

The small animal PET is an innovative preclinical imaging technique that is meant to visualise biological processes of tissues in a living organism. The most important characteristic is the very high spatial resolution that makes those tomographs suitable for imaging small animals like mice. The employment of different radiolabelled compounds allows to highlight the overexpression or nonexpression of many metabolic pathways, helping to profile in vivo the cancer from a biological point of view, to predict or measure the response to experimental therapies, to observe the metabolic modification of the cancer over time, and to test new labelled compound to be eventually used for clinical PET. Main drawback is the very high cost of the scanners and the need of a radiopharmacy, partly cyclotron based, to synthesise as many PET tracers as possible.

Contents

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1 Introduction

Currently, the most advanced techniques to evaluate molecular events in vivo come from nuclear medicine. Above all, PET (positron emission tomography) allows the quantification of different metabolic functions in organs and tissues and it is clinically widely used both for diagnostics and assessing therapy response in oncology, cardiology, and neurology.

Recently, the PET technology was introduced in the preclinical field since new small animal PET tomographs were implemented. This was possible thanks to new technologies (Wang et al. 2006) that are meant to enhance as much as possible the spatial resolution, therefore becoming appropriate for the evaluation of small-sized animals as rodents (Cherry 2006; Lewisa et al. 2002).

As for the clinical PET, in the preclinical field the PET imaging is addressed to the macroscopic visualisation of cellular processes by means of positron emitter radiolabelled compounds. This can be done with unspecific compounds that highlight general metabolic processes within the cells (e.g. ^{18}F-FDG, ^{11}C-Methionine etc.), with specific compounds (among which the receptor-tracers are the most commonly employed), or with the reporter-gene reporter-probe technique for cell tracking (Yaghoubi et al. 2006; Sossi and Ruth 2005).

The main advantage of microPET over the standard way of performing preclinical experiments (mainly based on the sacrifice of the animal) is the possibility to longitudinally follow the development of the disease (or the response to a specific new therapy) over time and in the same subject, significantly reducing the number of animals employed and increasing the reliability of the results. The high penetration power of the gamma rays created from the annihilation associated to the limited thickness of the rodents body allow a precise quantitation of the uptake even in deep anatomical structures (e.g., abdominal organs) and makes the microPET technology the most accurate tool to observe deeply located events. Furthermore, the microPET technology allows the detection of very low concentrations of tracers (picomolar order of magnitude), leading to an unequalled sensitivity in the visualisation and quantitative measurement of molecular processes (Sossi and Ruth 2005).

Another very interesting characteristic of the preclinical PET imaging is summarised in the word "translational". By employing the same technology in an experimental setting and in the clinical practice (in this case the PET imaging), in fact, the step between preclinical science and clinical applications in human patients becomes shorter and faster, reducing the overall time to effectively verify the clinical utility of the new approach. The most significant example in this field is the in vivo testing of new radiolabelled compounds meant to increase the specificity of the PET imaging for a specific disease. The creation of animal models of human diseases to test new compounds in vivo allows to avoid the translation into the clinical situation of those molecular imaging probes which do not bind selectively and with high affinity to the pathophysiological target molecules.

The translational applicability of this technique, the possibility to accurately quantify (in terms of time-activity curves, target to background ratio or standardised uptake ratio) (Myers and Hume 2002), the improved spatial resolution (that reaches 1 mm), the high sensitivity, the possibility to use targeted probes increasing the specificity of the metabolic information obtained from the scan, and the repeatability of the procedure on the same animal (a relatively limited dose is delivered to the animal for each scan) are features that make the microPET one of the most appreciated oncoming imaging technologies in the preclinical scenario, despite its relatively high costs related to the need of a cyclotron-based radiopharmacy.

2 Small Animal PET

2.1 General Aspects

Positron emission tomography (PET) is a nuclear medicine imaging technique which produces a three-dimensional image or picture of molecular processes in the body. The system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule. Images of tracer concentration in three-dimensional space within the body are then reconstructed by computer analysis. In modern scanners, this reconstruction is often accomplished with the aid of a CT X-ray scan performed during the same session, in the same machine.

If the biologically active molecule chosen for PET is ^{18}F-FDG, an analogue of glucose, the concentrations of the tracer imaged yield quantitative data on tissue metabolic activity, in terms of regional glucose uptake. Although use of this tracer results in the most common type of PET scan, other tracer molecules are used in PET to image the tissue concentration of many other types of molecules of interest.

To conduct the scan, a short-lived radioactive tracer isotope is injected into the living subject (usually into blood circulation) (Fig. 1) (Fueger et al. 2006). The tracer is chemically incorporated into a biologically active molecule. There is a waiting period while the active molecule becomes concentrated in tissues of interest; then the research subject is placed in the imaging scanner. The molecule most commonly used for this purpose is fluor-18-fluorodeoxyglucose (^{18}F-FDG), a sugar, for which the waiting period is typically one hour. During the scan a record of tissue concentration is made as the tracer decays.

As the radioisotope undergoes positron emission decay (also known as positive beta decay), it emits a positron, an antiparticle of the electron with opposite charge. After travelling up to a few millimetres the positron encounters an electron. The encounter annihilates them both, producing a pair of annihilation (gamma) photons moving in opposite directions. These are detected when they reach a scintillator in the scanning device, creating a burst of light which is detected by photomultiplier tubes or silicon avalanche photodiodes. The technique

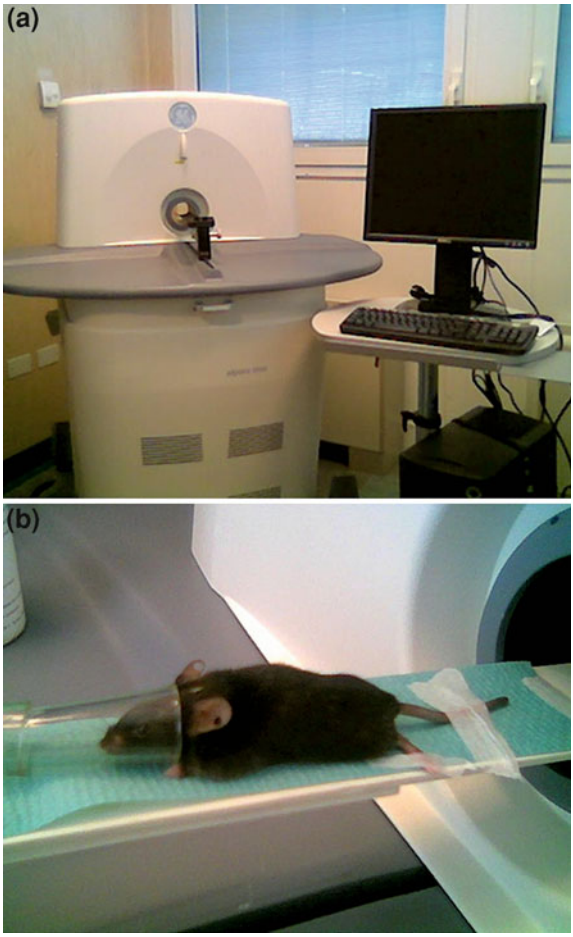

Fig. 1 **a** Small animal PET tomograph. **b** An animal model of cancer during gas anaesthesia on the scanner bed

depends on simultaneous or coincident detection of the pair of photons moving in approximately opposite direction (it would be exactly opposite in their centre of mass frame, but the scanner has no way to know this, and so has a built-in slight direction-error tolerance). Photons that do not arrive in temporal "pairs" (i.e., within a timing window of few nanoseconds) are ignored.

Radionuclides used in PET scanning are typically isotopes with short half-lives such as carbon-11 ($\sim$20 min), nitrogen-13 ($\sim$10 min), oxygen-15 ($\sim$2 min), and fluorine-18 ($\sim$110 min). These radionuclides are incorporated either into compounds normally used by the body such as glucose (or glucose analogues), water or ammonia, or into molecules that bind to receptors or other sites of drug action. Such labelled compounds are known as radiotracers. It is important to recognise that PET technology can be used to trace the biologic pathway of any compound in living organisms, provided it can be radiolabelled with a PET isotope. Thus, the specific processes that can be probed with PET are virtually limitless, and radiotracers for

new target molecules and processes are being synthesised all the time. Due to the short half-lives of most radioisotopes, the radiotracers must be produced using a cyclotron and a radiochemistry laboratory that are in close proximity to the PET imaging facility. The half-life of fluorine-18 is long enough such that fluorine-18-labelled radiotracers can be manufactured commercially at an offsite location.

2.2 Small Animal PET

In the literature, the vast majority of studies employing a small animal PET scanner are not based on the comparison between PET results and other preclinical imaging procedures but take into consideration histochemical analysis or autoradiography to verify imaging results. This is mainly due to the high costs of the scanners that make it very difficult to access a multiple modality technology. In the future this approach will change, and more and more complementary imaging techniques will be employed for evaluating the same animal model.

Small animal PET allows the non-invasive measurement of a range of tumour-relevant parameters at both the cellular and the molecular level that can be observed longitudinally over time. Studies to evaluate tumour response to a therapeutic intervention can achieve statistical significance using smaller groups of animals, as tumour cell physiology and tumour burden can be accurately determined pre- and post-therapy without assumptions.

Usually, the small animal PET imaging can be used for predicting tumoural cell engraftment (Fueger et al. 2006; Apisarnthanarax et al. 2006; Su et al. 2006; Hsueh et al. 2006; Hoekstra et al. 2000) in xenograft animal models, for assessing new therapies response in vivo, for the biological profiling of tumours by combining different tracers, for observing in vivo the metabolic behaviour of a tumour model over time, and for testing the accuracy of new PET compounds that eventually will be introduced into clinical practice.

The most widely employed PET imaging probe is ^{18}F-labelled glucose, which achieves tumour-specific accumulation on the basis that tumour cells have a higher rate of glucose uptake and metabolism (glycolysis) than normal tissues. ^{18}F-FDG is basically used in oncology to predict cancer cell engraftment (Nanni et al. 2007) and to measure the response to therapy. ^{18}F-FLT and its analogues (like ^{18}F-FMAU) are another family of compounds that are widely used in preclinical PET because they demonstrate the proliferative index of tumour masses with high accuracy, which is far higher in animal models than in patients (Sossi and Ruth 2005) (Fig. 2). ^{11}C-choline is a compound demonstrating the cell membrane proliferative activity, while ^{11}C-methionine highlights the rate of protein synthesis within the cells since it is an amino acid analogue.

Another small animal PET diagnostic method is the reporter-gene reporter-probe mechanism (HSV1—^{18}F-FHBG is the most validated) that allows the detection of genetically labelled viable cells injected into a living animal over a long time in vivo. This is particularly useful for tracking stem cells used for experimental and innovative regenerative therapies (Yaghoubi et al. 2006).

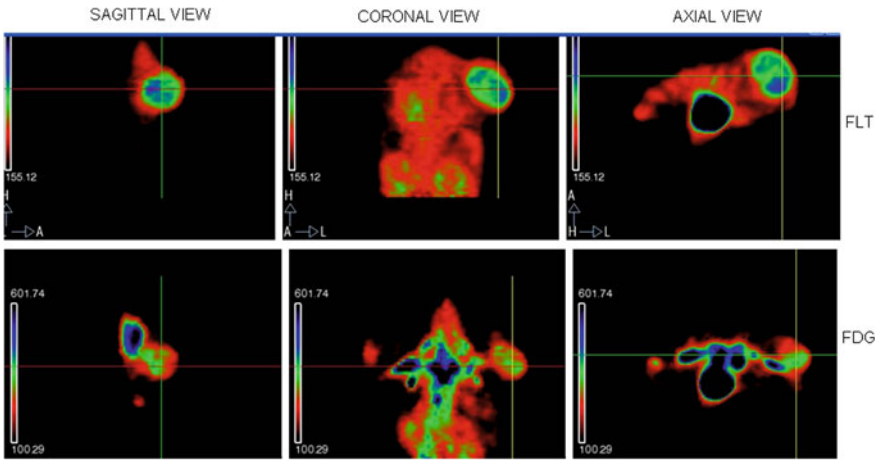

Fig. 2 Subcutaneous xenograft animal model of gastrointestinal stromal tumour evaluated with FLT and FDG PET. FLT images show the quote of proliferating cells while FDG images show the amount of glucose metabolism within the mass (reflecting the degree of malignancy)

Many other PET probes have been developed and are under development to obtain tumour specificity via a variety of tumour-specific mechanisms. The development of targeted radiolabelled ligands has further enabled PET to image many aspects of tumour biology in vivo. Radiolabelled annexin V, RDG peptide, VEGF, and $\alpha_v\beta_3$ integrin, for example, have been successfully tested in tumour models as well as in models of cardiac infarction, respectively, demonstrating apoptosis and perfusion (Cai et al. 2006; Liu 2006; Cauchon et al. 2007; Dobrucki and Sinusas 2005; Bauwens et al. 2011; Sherif et al. 2012; Cheng et al. 2011). The pharmacokinetics and pharmacodynamics of radiolabelled anticancer therapeutics can, in principle, also be monitored by these methods, thereby leading to rapid improvements in drug scheduling or design.

Finally, the effects of receptor therapies (e.g., inhibitors of androgen or oestrogen receptors or of epithelial growth factor receptor) can theoretically be predicted, thanks to the in vivo demonstration of the receptor after injection of the radiolabelled ligand (Myers and Hume 2002).

The literature includes a wide number of PET radiolabelled compounds for preclinical evaluation of specific molecular events, and it would be very difficult to provide a complete list of all the proposed compounds for oncological studies in the past decade.

2.3 Small Animal CT and Small Animal PET

The small animal CT can be used as a supporting method for small animal PET basically for three reasons. First of all it allows, as in the clinical setting, the correct anatomical localisation of PET findings. This is of particular interest in the

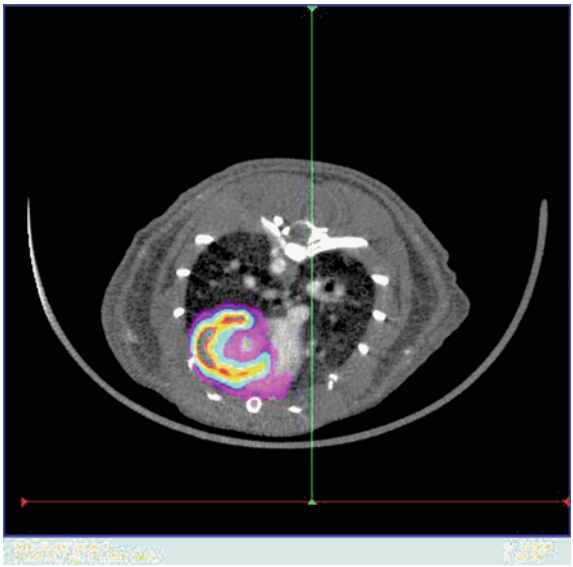

Fig. 3 Axial view of the fusion between FDG small animal PET and vascular contrast CT, showing the left ventricle of the heart

field of small animal imaging, because the use of experimental and very specific tracers prevents the PET from delineation of the animal shape. So, the PET image sometimes represents just a hot spot, whose anatomical localisation is impossible in absence of an anatomical reference (Fig. 3).

Second, the CT image can be used as an attenuation correction map, exactly as for clinical patients. Actually this is not of much interest, since the animal body is very small and therefore highly energetic photons are subjected to a negligible attenuation, but it can be important when studying bigger animals like primates to achieve a correct quantification of PET tracer uptake.

Last, but not the least, CT images can be very useful for integrating the metabolic results obtained by the PET. The CT can be used, for example, to exactly measure the organ sizes or the tumour diameters, and these parameters can be noninvasively monitored over time. The CT provides a density map and can be very useful to diagnose, for example, the onset of necrosis, of small liver nodules, of ascites, and so on. However, the bigger contribution of small animal CT is in the field of bone structure evaluation (osteoporosis, osteomyelitis, and fractures) and lung evaluation.

Recently, the technological development led to the possibility to acquire CT images with cardiac gating and respiratory gating. While the respiratory gating is aimed just to the improvement of spatial resolution at the base of the animal lungs, the cardiac gating has much more scientific importance. In fact, it can be used as an alternative method to measure the ejection fraction for cardiac studies, also because it is possible to intravenously inject vascular contrast agents helping in the visual differentiation between the ventricular wall and the ventricular cavity. These data can be combined with all the PET metabolic results that have been already explained in the previous paragraphs.

Most of the published studies regarding the small animal PET-CT imaging are performed by using two separate scanners. This is possibly due to the introduction of multimodality beds on the market that can be shifted from one scanner to the other with the same subject on. In this way the position of the experimental animal does not change (or at least is subjected only to minimal variations) and the two image sets (usually in DICOM format) can be subsequently co-registered with specific software.

It is important to point out that, to co-register a PET and a CT image, it is necessary to have at least three reference points, aimed at guaranteeing a slice by slice correct alignment. These reference points must be external to the animal, radioactive for PET imaging, radio-opaque for CT imaging, and must be positioned for example on the multimodality bed before starting the imaging session.

Despite, as explained, the acquisition image technique may be quite complicated in practice when two separate scanners are used, after a short training very high quality images can be obtained, especially if a sufficiently long anaesthesia is provided to the animal.

3 Conclusion

The preclinical functional imaging (in particular small animal PET) is a niche but an important field of preclinical basic research. It allows to observe the development of a specific disease on animal models in vivo, to highlight their molecular profile, to evaluate their response to new experimental therapies in vivo, and to test new radiolabelled compounds for the clinical PET imaging. In this way the step from bench to bedside may become shorter, accelerating the introduction of new therapies or imaging methods in the clinical practice.

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