# 12 Pharmacological Prerequisites for PET Ligands and Practical Issues in Preclinical PET Research

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**Abstract.** The development of PET radiopharmaceuticals for the non-invasive imaging of cancerous lesions, brain receptors, transporters and enzymes started more than 25 years ago. But till today no established algorithms exist to predict the success of a PET radiopharmaceutical. PET radioligand development is a challenging endeavor and predicting the success of PET ligand can be an elusive undertaking. A large number of PET radiopharmaceuticals have been developed for imaging, but so far only a few have found application as imaging agents in vivo in humans. Typically, the potential compound selected for development usually has the desired in vitro characteristics but unknown in vivo properties. The purpose of this chapter is to highlight some of the pharmacological constraints and prerequisites. Interspecies difference in metabolism and mass effects are discussed with examples. Finally, some of the practical issues related to laboratory animal imaging using anesthetic agents are also presented.

## 12.1 Introduction

Positron emission tomography (PET) is an imaging modality that allows study of physiological, biochemical and pharmacological functions at the molecular level. The development of PET radiopharmaceuticals for the non-invasive imaging of cancerous lesions, brain receptors, transporters and enzymes started more than 25 years ago, but till today no established algorithms exist to predict the success of a PET radiopharmaceutical. Driven by new post-genomic techniques such as proteomics, genomics and protein–protein interactions, pharmaceutical companies have developed and continue to develop a large number of drugs for various targets. The general assumption is for the most part that drugs which are fetching money for the pharmaceutical companies would also be excellent PET radiopharmaceuticals if amenable to labeling with PET isotopes. This assumption is not necessarily true. For example, the antidepressant fluoxetine (Prozac), although performing well on the drug market as a pharmaceutical, is due to high non-specific binding in vivo unsuitable as a PET ligand for imaging the serotonin transporter (Shiue et al. 1995). PET radioligand development is a challenging endeavor and predicting the success of PET ligand can sometimes be an elusive undertaking. A large number of PET ligands have been developed for imaging, but so far only a few have found application as imaging agents in vivo in humans. As with drug development, there are various stages involved in the PET radiopharmaceutical development process starting from candidate compound selection through to human application. Typically, the potential compound selected for development usually has the desired in vitro characteristics such as high affinity, selectivity and other appropriate pharmacologic properties for the target under investigation. But factors such as in vivo affinity, metabolic stability and pharmacokinetics are for the most part unknown and contribute to a great extent to the failure of PET radiopharmaceuticals. The question that arises is which in vivo parameters, criteria or factors should one be aware of or carefully consider when developing PET radiopharmaceuticals in order to reduce attrition in the PET radiopharmaceutical development process. Ideally, PET radiopharmaceuticals should fulfill several key requirements. The ligand should have high affinity and selectivity to provide an adequate target-to-non-target ratio. The labeled compound should be produced in

high specific radioactivity and for PET radiotracers of the central nervous system (CNS) metabolism should not yield radioactive metabolites that cross the blood–brain barrier. The compound should be non-toxic and not have affinity for efflux pumps.

This chapter describes with illustrative examples some major pharmacological properties of PET radiotracers as well as practical issues which should be considered during the development and validation phase of a new PET radiopharmaceutical.

## 12.2 Pharmacological Constraints

PET imaging studies generally use tracer doses containing only very small amounts of the radiopharmaceutical (in the range of picograms to micrograms) which conform to true tracer kinetics and have only marginal or negligible pharmacological effects and toxicity. The limited sensitivity of small animal PET imaging systems compared with clinical tomographs requires a higher number of coincidence events detected from a rodent imaging voxel to achieve a comparable count statistics as in human PET studies. Therefore, significantly higher doses of radioactivity have to be administered to a rodent. Such an approach, however, may be associated with the injection of a considerable mass of stable compound.

The impact of administrating high doses to mice and rats is closely related to the target protein density or capacity and the affinity of the tracer. Depending on the mechanism of uptake and retention, PET radiopharmaceuticals can be classified in three main classes (Jagoda et al. 2004): (1) non-saturable systems (e.g., [$^{18}$F]-fluoride uptake in bone), (2) intermediate saturable systems (e.g., [$^{18}$F]-FDG measuring GLUT-1 capacity and hexokinase activity), and (3) saturable binding sites, typically receptor binding sites with low density (e.g., [$^{18}$F]-fallypride binding to D2 receptors).

Tracers belonging to class 1 pose the least challenges; their uptake in the target region will not be affected even at very high injected doses. For class 2 tracers such as [$^{18}$F]-FDG, the situation may already become more problematic when glucose levels in the blood are high and the saturation level of the hexokinase is potentially reached (Jagoda

et al. 2004). The hypoxia tracer [$^{18}$F]-FMISO represents a comparable case to [$^{18}$F]-FDG. [$^{18}$F]-FMISO undergoes reduction in hypoxic tissue requiring intact nitroreductase enzymes and it is still a matter of debate whether the nitroreductase enzymes become saturated depending on the injected mass of unlabeled FMISO and therefore cause a non-oxygenation-related reduction in radioactivity uptake in hypoxic tissue even though a 'no-carrier-added' method is used for the preparation of the radiotracer. The quantification of [$^{18}$F]-FMISO uptake in B16 melanoma-bearing mice using injectants with escalating amounts of unlabeled FMISO demonstrated that even co-injection of 'no-carrier-added' [$^{18}$F]-FMISO with up to a thousand-fold excess of cold FMISO did not show any significant correlation between [$^{18}$F]-FMISO uptake and injected mass of FMISO (Fig. 1).

For class 3 radiotracers, the target saturation can be easily assessed by in vivo small animal imaging studies or ex vivo dissection techniques. Using escalating carrier-added doses, the ligand dose which is blocking 50% of specific binding ($ED_{50}$) can be determined. The $ED_{50}$ value was

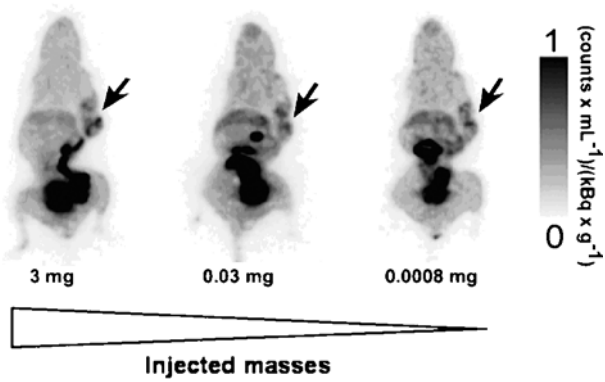


**Fig. 1.** The same B16 melanoma-bearing mouse scanned on three different days after [$^{18}$F]-FMISO injection with a decreasing injected mass of unlabeled FMISO. Upon visual inspection, no difference in radiotracer uptake or image quality is apparent. The tumor is marked by an arrow. (Modified from Wyss et al. 2005)

found to be unpredictable from the binding potential ($BP = B_{max}/K_d$) as measured by in vitro binding assays (Hume et al. 1995). The discrepancy of in vitro and in vivo values may be explained by factors such as non-specific binding, soft tissue retention, metabolism, blood–brain barrier penetration, and micro-environment of the target site. Therefore, the term 'apparent' $K_d$ for values determined in vivo is often used to reflect these differences between both types of analyses (Kung et al. 2005). Especially for high-affinity PET tracers ($K_d$ <0.1 nM) involved in easily saturated processes (e.g., ligand–receptor interactions) the specific activity of the radiotracer plays an important role and can limit its usefulness as an imaging agent. Even small amounts of injected tracer may lead to a significant receptor occupancy which is associated with a deterioration of signal-to-noise ratios, the violation of kinetic modeling assumptions (postulating less than 5% receptor occupancy), and – depending on the target and its effector system – with pharmacological or toxic effects. Even for PET tracers with intermediate affinity such as [$^{11}$C]-raclopride ($K_d$=1 nM) or with low affinity (e.g., [$^{11}$C]-CFT; $K_d$=10 nM) a considerable degree of receptor occupancy may be reached (Hume et al. 1995). For future studies, increasing the specific activity of the radiotracer and the sensitivity of the small animal PET system may be crucial to avoid conflicts with this pharmacological constraint.

## 12.3 Radiotracer Metabolism

In general, the difficulty in predicting metabolism of a new candidate ligand stems from the fact that numerous factors such as enzyme expression, interspecies differences, inter-animal or inter-individual variability, age and hormonal status all play a role in determining the metabolic fate of a drug (Delaforge 1998). For CNS PET radiotracers, as mentioned above, radiolabeled metabolites should not enter the brain and confound the PET signal. 6-[$^{18}$F]-Fluoro-L-DOPA ([$^{18}$F]-FDOPA) is one of the most widely used PET tracers for studying presynaptic dopaminergic function in Parkinson's disease (PD). In the brain, [$^{18}$F]-FDOPA is decarboxylated to [$^{18}$F]-Fluoro-L-dopamine and like dopamine accumulates in dopaminergic terminals. Metabolic studies showed that besides [$^{18}$F]-Fluoro-L-dopamine, other radiolabeled metabolites are

also formed which cross the blood–brain barrier and contribute to non-specific binding in the brain. As such for an accurate analysis of [$^{18}$F]-FDOPA uptake the determination of [$^{18}$F]-FDOPA and its radioactive metabolites in blood plasma is essential. Despite this shortcoming, [$^{18}$F]-FDOPA has proven its utility in the clinical diagnosis of PD. We evaluated the possibility of using [$^{18}$F]-FDOPA in a mouse brain and found that unlike in humans and primates [$^{18}$F]-FDOPA was only uniformly distributed in mouse brain with no specific accumulation in the striatum (Fig. 2) despite the concomitant use of aromatic amino acid decarboxylase (AADC) and catechol-*O*-methyltransferase (COMT) inhibitors (Honer et al. 2005). Hume and colleagues reported similar findings in rat brain using [$^{18}$F]-FDOPA (Hume et al. 1996). The use of [$^{18}$F]-FMT (6-fluoro-meta-tyrosine), a structural analogue that is not a substrate of COMT, also did not provide a useful image contrast (Fig. 2).

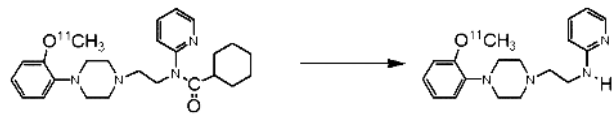


**Fig. 2.** Metabolism of [*O*-methyl-$^{11}$C]-WAY100635 in primates

Another illustrative example is the serotonin 5-HT$_{1A}$ antagonist [*O*-methyl-$^{11}$C]-WAY100635 which was identified as the first useful radioligand for visualizing the 5-HT$_{1A}$receptors in rodents. In rats, the radioactive metabolites formed were more polar than the parent compound and did not enter rat brain (Pike et al. 1995). However, PET and plasma metabolic studies in humans and primates indicated that a different radiolabeled metabolite, the des-cyclohexanecarbonyl derivative [*O*-methyl-$^{11}$C]-WAY100634 (Fig. 3) was formed that passed into the brain and contributed to specific and non-specific binding (Osman et al. 1996). Further optimizations eventually led to the development of more metabolic stable compounds which are currently being used to study the 5-HT$_{1A}$ receptors in humans. The lessons from $^{18}$F-FDOPA and [*O*-methyl-$^{11}$C]-WAY100635 metabolism remind us that interspecies differences in radioligand metabolism should always be taken into account and wrong decisions could be taken when the radioligand is re-

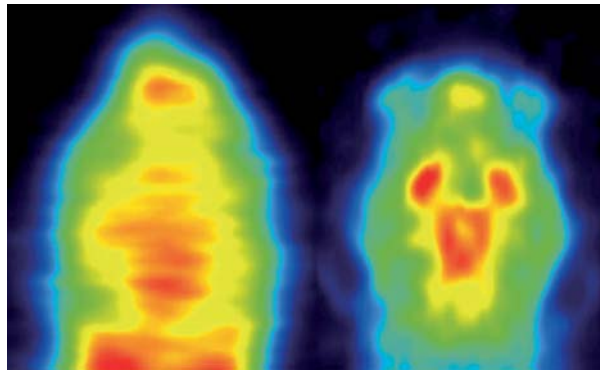

**Fig. 3.** Comparison of representative coronal PET images through the brains of two different C57/BL6 mice injected with [$^{18}$F]-FDOPA (reconstructed data from 15–45 min p.i.) and [$^{18}$F]-FMT (reconstructed data from 88–128 min p.i.). The plane shown passes through the level of the striatum

jected too early or too late when no early information on potential metabolism problems in humans is available. In vitro metabolism studies using rat or human hepatocytes in combination with high-pressure liquid chromatography and mass spectrometry can very well predict in vivo metabolism as demonstrated recently by Ma and colleagues (Ma et al. 2003).

## 12.4 Practical Constraints: Anesthesia

Small animal PET imaging involves the necessity to immobilize the animal during the scanning procedure. The immobilization is generally accomplished using anesthetics which, however, affect physiological and hemodynamic parameters of the animal. Therefore, tracer uptake, kinetics and metabolism may be influenced in vivo by the use of anesthesia, thus complicating data interpretation of animals under anesthetized conditions. Particularly, PET imaging using radiotracers that are rapidly metabolized in vivo will be flawed by anesthesia.

Several issues have to be considered to reduce the confounding influence of anesthesia on the function of interest. In order to minimize

the effects on animal physiology, anesthesia should be administered in a controllable manner and at a superficial level. For such an anesthesia, the most consistent and convenient regimen in small animal PET imaging involves inhalation anesthetics and spontaneously breathing animals. Isoflurane is the most commonly used inhalation anesthetic in small animal imaging, but is also known to have some effects on regional cerebral blood flow and brain glucose metabolism (Fig. 4). Depending on the PET tracer, scanning protocol and function of interest, the ideal anesthetic regime should be selected for each experimental protocol. In addition, for each tracer employed in small animal PET scanning it should be considered whether the anesthetics may directly or indirectly interact with the target site of the tracer (Votaw et al. 2003; Opacka-Juffry et al. 1991; Tsukada et al. 1999). Apart from isoflurane inhalation anesthesia, other commonly used anesthetics in small animal imaging are pentobarbital, α-chloralose, propofol and ketamine/xylazine.

An optimal anesthetic protocol also involves reliable monitoring of the depth of anesthesia using the respiratory frequency as the best monitoring parameter. Homeostasis of fluid and electrolyte balance, acid/base balance and blood glucose levels by fluid supplementation during anesthesia may also become very important, especially for long-term scanning (>30 min). In addition, body temperature should be controlled by a rectal probe and an automated feedback temperature control system

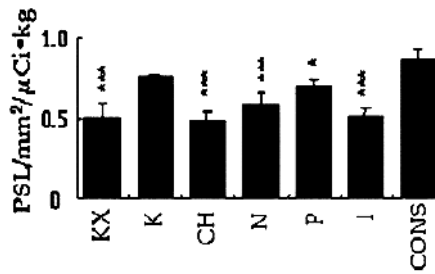


**Fig. 4.** Average normalized activity uptake in whole brain of rats injected with [$^{18}$F]-FDG and anesthetized with various regimen (*KX*, ketamine/xylazine; *K*, ketamine; *CH*, chloral hydrate; *N*, pentobarbital; *P*, propofol; *I*, isoflurane; *CONS*, controls). (Adapted from Matsumura et al. 2003)

connected to an appropriate heating device. Precise monitoring of anesthesia is also crucial for the maintenance of physiological and hemodynamic stability within and across PET studies, thus increasing the intrastudy and interstudy reproducibility of PET data.

PET studies in rodents without anesthesia remain challenging for small animal PET experimentation. In a recent study, a non-anesthetized rat was trained to accept head fixation and scanned for 1 h (Momosaki et al. 2004). However, the effects of physical and mental stress imposed on the animal by active restraint have to be analyzed. Furthermore, the compromising effects of anesthesia can be largely avoided for tracers that are efficiently trapped in their target compartment. The animal is then allowed to remain awake during uptake and accumulation of the radioactive probe and is anesthetized only at a later time point when tracer uptake is complete and a steady state has been reached. [$^{18}$F]-FDG is a good example of a tracer that may be used in such a manner (Matsumura et al. 2003).

## 12.5 Conclusions

In conclusion, PET radiopharmaceutical development is an iterative process, requires a bit of luck and it can sometimes be a trial and error exercise. One may follow all the guidelines and criteria needed for a successful ligand but there is no guarantee that a successful tracer for human application will be the end product and obviously no successful radiopharmaceutical can fulfill all the requirements.

Suitable animal models may help to facilitate PET tracer development. For example, animals with a targeted disruption of a specific gene can be elegantly used to demonstrate in vivo specificity of tracer binding to the target gene product (Ametamey et al., 2006). However, the validity of each animal model (e.g., murine models for Alzheimer's or Parkinsons's disease) for tracer development has to be carefully evaluated in advance.

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