Introduction on PET

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Introduction on PET: Description of Basics and Principles

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Abstract

Positron emission tomography (or PET) is a non-destructive imaging technique in nuclear medicine with several unique properties: high sensitivity, low radiation dose, possibility to correct data for attenuation and scatter (thus quantitative), radioactive labeling of natural substances or drugs with high specific radioactivities so that these can be used as tracers to monitor the pharmacokinetics of the non-radioactive compounds. Limitations are the spatial resolution of commercially available PET cameras, resulting in blurring or non-visibility of objects smaller than 1 mm, and the short half-lives of the commonly used PET radionuclides (< 2 h). Because of the combination of positron emitters, specific radiopharmaceuticals and quantitative data analysis, PET is frequently used to study the pharmacokinetics and pharmacodynamics of test drugs non-invasively in humans.

Positron Emission Tomography or PET is a medical imaging technique providing information on tissue biochemistry rather than anatomy (Paans *et al.*, 2000; Cherry, 2001; Levin, 2005; Wang *et al.*, 2005).

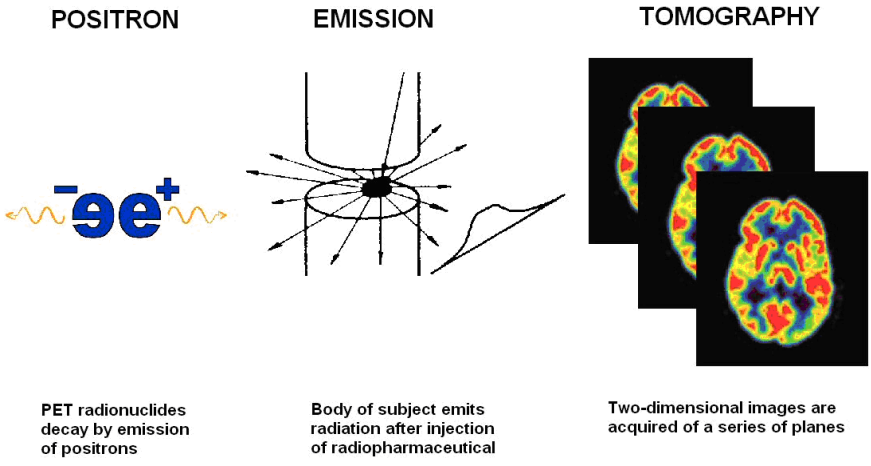

Fig. 1 Explanation of the term "positron emission tomograpy".

The term *tomography* indicates that two-dimensional images are acquired of several adjacent cross-sections ("planes" or "slices") within the subject's body (Fig. 1). The word *emission* shows that these images are generated after intravenous injection of a radioactive compound (the "radiopharmaceutical") into the volunteer or the experimental animal (Fig. 1). The radiopharmaceutical is transported throughout the body by the circulation and taken up by target organs resulting in the emission of externally detectable radiation. The emitted radiation is measured by a PET camera and used for imaging. The radiopharmaceuticals which are used in PET are marked with radionuclides which decay by the emission of *positrons* (Fig. 2). Positrons are anti-electrons, i.e. particles with the same mass as an electron but a positive in stead of a negative charge. This form of radioactive decay is important both for image reconstruction and for the interpretation of PET images.

A PET camera does not detect the positrons themselves for their range of travel within tissue is too short (less than 2.5 mm). Rather, the camera detects the two gamma quanta which originate from the *annihilation* of these anti-electrons. When a positron meets a normal electron, the combined mass of the two particles is converted to energy in a process that is called annihilation. Annihilation of a

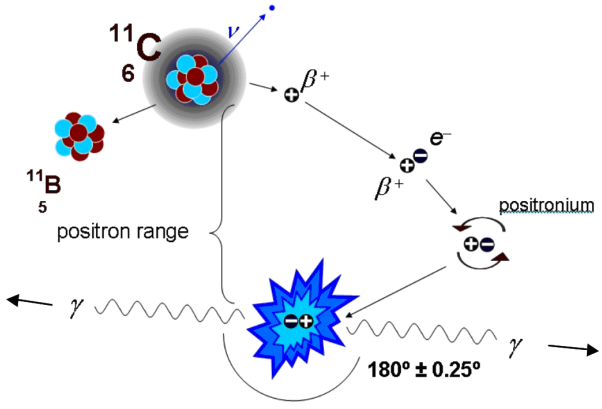

Fig. 2 Radioactive decay of ^{11}C and positron annihilation.
(Illustration provided by Dr. J. Carney, Dept. Radiology, University of Pittsburgh.)

positron results in the formation of two 511 keV photons which are emitted in anti-parallel (i.e. approximately 180 degrees opposite) directions (Fig. 2). These high-energy photons leave the body and are counted by detectors in the PET camera. Electronic circuits connected to the detector rings ensure that events are only registered when two detectors are (virtually) simultaneously hit by a photon. This *coincidence detection* has important advantages: (1) a low background count rate and a good signal-to-noise ratio of the detector system; (2) no collimator (i.e. no lead shield with narrow holes) is required for position information, resulting in a high sensitivity of PET cameras compared to normal gamma cameras using single photon emitters; and (3) images can be corrected for attenuation and scatter of the radiation within tissue. This correction is accomplished by making a transmission scan of the subject (patient, volunteer or experimental animal), using an external ($^{68}Ge/^{68}Ga$) gamma source, or by making a CT scan. Emission images (acquired after injection of the radiopharmaceutical) can then be corrected for attenuation measured with the external source, on the condition that the subject does not move between acquisition of the transmission (or CT) and emission scans. Thus, biochemical and physiological processes can be measured *quantitatively.*

A fundamental limitation in the spatial resolution of PET images is the range of positrons within tissue (Levin *et al.*, 1999;

Phelps *et al.*, 1975; Tai *et al.*, 2005). This varies from less than 1 mm for ^{18}F to 2.5 mm for ^{15}O, at the mean positron energy which is 40% of the maximum. Since the annihilation occurs at another location than the positron decay, the range traveled by the positron makes it impossible to estimate the decay position with absolute precision.

Since the elements carbon, nitrogen and oxygen have positron-emitting isotopes (^{11}C, ^{13}N and ^{15}O, respectively), drugs and biomolecules can be labeled with a positron emitter without changing their chemical properties. These isotopes can also be prepared with high specific radioactivities. Metabolites, neurotransmitters, hormones and drugs can therefore be labeled with positron emitters and be used as *tracers* to monitor the pharmacokinetics of the endogenous substance (or the non-radioactive test drug). A high specific radioactivity is particularly important in the case of receptor ligands, enzyme inhibitors or transporter blockers binding to a limited number of sites.

The radionuclides which are commonly used in PET have very short half-lives (^{15}O: two minutes, ^{13}N: ten minutes, ^{11}C: 20 minutes, ^{18}F: 109.8 minutes, respectively). Transport of carbon-11, nitrogen-13 and oxygen-15 over large distances is therefore impossible. Such radionuclides should be produced locally. ^{11}C, ^{13}N, ^{15}O and ^{18}F are made by nuclear reactions (Table 1). ^{11}C, for example, is made by irradiating nitrogen gas with protons. Protons of the right energy evoke the following nuclear reaction:

$$^{14}\text{N}\ (\text{p},\alpha)\ ^{11}\text{C}$$

The particles (protons or deuterons) causing the reaction are accelerated by means of a cyclotron. Small, dedicated medical cyclotrons are

Table 1 Radionuclides for PET.

Nuclide	*Half-life (min)*	*Decay product*	*Nuclear reaction*	*Theoretical specific activity (Ci/mmol)*
^{11}C	20.4	^{11}B	^{14}N (p,α) ^{11}C	9.22×10^6
^{13}N	9.96	^{13}C	^{16}O (p,α) ^{13}N	1.89×10^7
^{15}O	2.1	^{15}N	^{14}N (d,n) ^{15}O	9.08×10^7
^{18}F	109.8	^{18}O	^{18}O (p,n) ^{18}F	1.71×10^6

commercially available. In combination with sophisticated targetry, they can produce ^{11}C, ^{13}N, ^{15}O and ^{18}F in large amounts. In a cyclotron target, only simple molecules are formed. When the nitrogen gas used for production of ^{11}C is mixed with a trace of oxygen, the end product of the reaction is ^{11}C-carbon dioxide. Such simple building blocks (e.g. $^{11}CO_2$, ^{11}CO, $^{13}NH_4^+$, $^{13}NO_3^-$, $^{13}NO_2^-$) are used to synthesize the desired radiopharmaceuticals (see Table 1).

Since the half-lives of positron-emitting radionuclides are short, only rapid labeling procedures can be employed for radiopharmaceutical production. Radiochemists are responsible for these production processes. Many compounds are labeled by a single-step reaction of a des-methyl precursor with ^{11}C-methyl triflate or ^{11}C-methyl iodide. The final product then contains a ^{11}C-labeled methoxy or N-methyl group. Fluorine substitutions on an aromatic ring using ^{18}F-fluoride are also frequently employed for labeling. Synthesis modules for the automated production of common radiopharmaceuticals (e.g. ^{18}F-FDG, ^{18}F-FLT, ^{18}F-DOPA, ^{13}N-ammonia, ^{11}C-acetate and labeled substances produced by ^{11}C-methylation) can be purchased from several suppliers.

The short half-lives of positron emitters can be considered as a disadvantage of PET, since a local cyclotron and facilities for radiopharmaceutical production are required and biological processes with slow kinetics (e.g. the binding of antibodies to their targets) cannot be examined. However, the rapid decay may also be considered as an advantage. Relatively high doses of a positron emitter (several hundreds of MBq) can be administered to a subject, allowing the acquisition of images with good counting statistics for about three half-lives. Because of the short half-life of the employed radioactivity, the total radiation burden for the subject (i.e. the integral of radioactivity over time) remains low. The radiation burden for an average PET scan is 1 to 3 mSv, an order of magnitude lower than the average radiation burden for a CT scan (10 to 20 mSv). PET scans can therefore be made in healthy volunteers and subjects can be scanned repeatedly, e.g. at baseline and at two different time points after ingestion of a test drug (see Fig. 3).

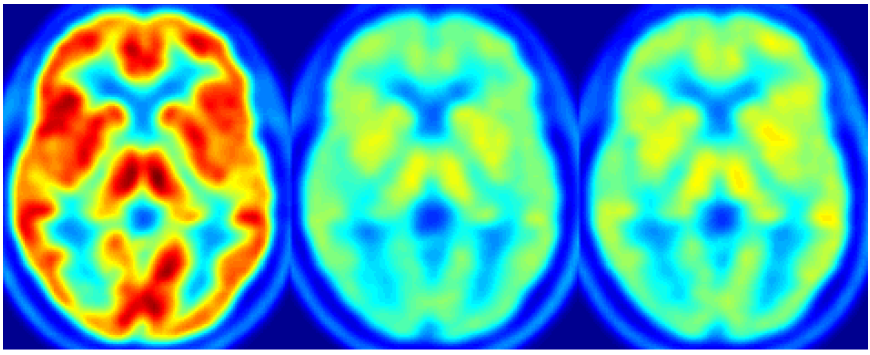

Fig. 3 Example of a receptor occupancy study performed in our institution. Three scans of the brain of a healthy volunteer were made, using the sigma-1 receptor ligand ^{11}C-SA4503: (a) at baseline (left image); (b) 3 h (middle image); and (c) 21 h (right image) after oral administration of an unlabeled sigma-1 agonist. Receptor occupancy was high (> 80%) after 3 h and showed only a minor decrease during the subsequent 21 h.

Each PET study requires the harmonious cooperation of chemists, pharmacists, physicists and physicians. The scanning protocol proceeds in a number of logical steps: (1) production of radioactivity; (2) preparation of the desired radiopharmaceutical; (3) purification, sterilization and quality control; (4) administration of the tracer to the experimental animal or the human subject; (5) data acquisition; (6) image reconstruction; (7) analysis of the data with tracer-kinetic models; and (8) reporting to the investigator or the physician of the patient.

Many physiological and biochemical processes can be quantified with a PET camera (see Table 2). These include: blood flow (tissue perfusion), blood volume in a region of interest, glucose consumption, amino acid transport, lipid metabolism (activity of choline kinase), oxidative metabolism (Krebs cycle activity), the rate of synthesis of various neurotransmitters (e.g. dopamine, serotonin), the regional distribution and density of many receptors (e.g. binding sites for dopamine, noradrenaline, serotonin, acetylcholine, bombesin, neurokinin), biomarkers of cellular proliferation (e.g. EGF receptor density, sigma receptor density, rate of incorporation of nucleosides

Table 2 PET tracers available in our institution.

Radiopharmaceutical	*Process visualized*	*Treated tissue/organ*
^{15}O-Water	Blood flow	Brain, heart
^{13}N-Ammonia	Blood flow	Heart
^{15}O-Carbon Monoxide	Blood volume	Brain, heart, lungs, tumors
^{18}F-Fluorodeoxyglucose	Glucose consumption	Brain, heart, tumors, muscle
^{11}C-Methionine	Amino acid transport	Tumors
1-^{11}C-Tyrosine	Amino acid transport	Brain, tumors
^{11}C-Choline	Choline kinase activity	Tumors
^{11}C-Acetate	Oxidative metabolism	Heart
^{18}F-Fluoro-L-DOPA	Dopamine synthesis rate	Brain, neuroendocrine tumors
^{11}C-5-Hydroxytryptophan	Serotonin synthesis rate	Brain, neuroendocrine tumors
^{11}C-Raclopride	Dopamine D_2/D_3 receptors	Brain (striatum)
^{11}C-N-propyl-norapomorphine	D_2/D_3 receptor (high affinity state)	Brain (striatum)
^{11}C-CGP12388	Beta-adrenoceptors	Heart, lungs, spleen
^{18}F-Fluorocarazolol	Beta-adrenoceptors	Heart, lungs, brain (animals)
^{18}F-MPPF	5-HT_{1A} receptors	Brain
^{11}C-VC002	Muscarinic cholinoceptors	Heart, lungs
^{11}C-VC003	Muscarinic cholinoceptors	Brain
^{11}C-SA4503	Sigma-1 receptors	Brain, tumors
^{18}F-FE-SA5845	Sigma-1/2 receptors	Brain, tumors
^{11}C-SA5845	Sigma-1/2 receptors	Brain, tumors
^{11}C-PK11195	Translocator protein	Brain (activated microglia)
^{11}C-DPA713	Translocator protein	Brain (activated microglia)
^{18}F-Bombesin	Bombesin receptors	Tumors (prostate)
^{18}F-FES	Estrogen receptors	Tumors (breast)
^{18}F-Substance P	Neurokinin-1 receptors	Lungs, tumors
^{18}F-FAZA	Hypoxia	Tumors
^{11}C-Meta-hydroxy-ephedrine	Noradrenalin transporter activity	Heart
^{18}F-FLT	Thymidine kinase-1 activity	Tumors
^{18}F-FHPG	Viral thymidine kinase	Brain, tumors (gene therapy)
^{18}F-FHBG	Viral thymidine kinase	Brain, tumors (gene therapy)
^{18}F-FEAU	Viral thymidine kinase	Brain, tumors (gene therapy)
^{11}C-MP4A	Acetylcholinesterase activity	Brain
^{11}C-Verapamil	P-glycoprotein function	Brain, tumors
^{11}C-Carvedilol	P-glycoprotein function	Brain, tumors
^{11}C-GR218231	P-glycoprotein function	Brain, tumors
^{11}C-PIB	Beta-Amyloid plaques	Brain

(*Continued*)

Table 2 (*Continued*)

Radiopharmaceutical	*Process visualized*	*Treated tissue/organ*
^{18}F-Desbromo-DuP697	Cyclooxygenase-2 expression	Brain, tumors
^{11}C-VIOXX	Cyclooxygenase-2 expression	Brain, tumors
^{124}I-Iodide	Thyroid imaging	Thyroid
^{18}F-Interleukin-2	Insulitis	Pancreas
^{89}Zr-Bevacizumab	VEGF levels	Tumor
^{89}Zr-Cetuximab	Epidermal growth factor receptor	Tumor
^{89}Zr-Trastuzumab	HER2/neu expression	Tumor

into cellular DNA), biomarkers of inflammation (e.g. translocator protein density, binding sites for interleukin-2), the expression levels and activity of various transporters (e.g. P-glycoprotein, noradrenalin transport, iodine transport), and the activity of important enzymes (e.g. thymidine kinase, cyclooxygenase-2). Using specific radiopharmaceuticals, PET can be applied in various ways during the drug discovery process.

First, PET has important applications in *pharmacodynamics*. Pharmacodynamics is the study of the effects of drugs in the healthy and diseased body, including the mechanism of action and the relationship between drug concentration and effect. PET is capable of measuring the metabolic response of tissues in the human body quantitatively, repeatedly and noninvasively which can speed up drug development and can significantly reduce the associated costs. In oncology, PET may be employed to assess the effectiveness of novel anti-cancer agents and to optimize therapeutic protocols, e.g. by measuring the effect of the drug on glucose consumption, amino acid uptake or nucleoside metabolism within tumor cells (Avril *et al.*, 2007; Kumar *et al.*, 2007; Aboagye *et al.*, 2003; Boss *et al.*, 2008). During the development of CNS drugs, PET is often used to measure the dose-dependent occupancy of target receptors within the human brain by a non-radioactive test compound (van Waarde, 2000; Passchier *et al.*, 2002; Wong *et al.*, 2009; Talbot *et al.*, 2002; Halldin *et al.*, 2001)(see Fig. 3). Employing the

radioligand/unlabeled competitor approach, such studies can answer the following questions:

(1) Does the unlabeled drug reach its intended target?
(2) How is occupancy of the target protein related to the administered dose?
(3) What is the relationship between target occupancy and plasma kinetics of the drug?
(4) Which level of receptor occupancy is required for the beneficial effect?
(5) At which threshold level of occupancy do unwanted side effects arise?
(6) What is the optimal dose regime (beneficial effect reached but side effects largely avoided)?
(7) Is occupancy of the target in non-responders lower than in responders, or do other factors preclude the beneficial effect?

By studying only a limited number of volunteers, these questions can be answered and the optimal dose regimen for further clinical studies can be selected.

Second, if the test drug itself can be labeled with a positron emitter, PET may be employed to study the *pharmacokinetics* of a novel drug candidate. After intravenous injection, inhalation or ingestion of the labeled compound, the time-dependent concentration of radioactivity in different organs and tissues can be measured quantitatively (Klimas, 2002; Hutchinson *et al.*, 2003; Fischman *et al.*, 2000; Berridge *et al.*, 2000) (see Fig. 4). Since the drug is labeled with a high specific radioactivity, only minute doses of the test compound need to be administered for accurate measurements with a PET camera. This "microdosing" approach facilitates PET studies in human subjects at an early stage of drug development (Bauer *et al.*, 2008; Wagner *et al.*, 2008). The role of the blood-brain barrier in drug delivery to the brain can be assessed, e.g. by measuring brain uptake of the test compound in the presence and absence of a modulator of P-glycoprotein (Elsinga *et al.*, 2004). Various strategies for drug delivery can be compared, e.g. various liposome formulations (Oku *et al.*, 2000). The effect of specific interventions on

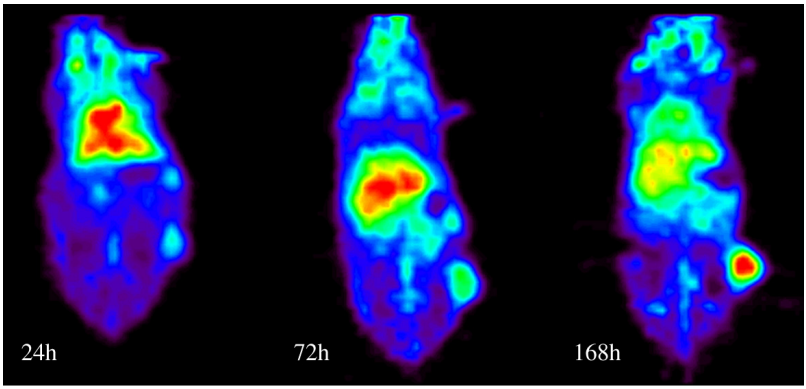

Fig. 4 Example of a PET study of drug pharmacokinetics. We labeled a tumor-targeting antibody with the positron emitter ^{89}Zr (half-life 3.27 d) and injected it into a tumor-bearing mouse. Whole-body microPET scans were made of this animal at 24 h, 72 h and 168 h after injection. The contrast between the tumor (implanted into the right hindleg) and non-target organs showed a steady increase during the 168 h period.

pharmacokinetic parameters can also be examined, e.g. the impact of inhibition of the plasma protein binding (Haradahira *et al.*, 2000), metabolic degradation (Tipre *et al.*, 2006; Doudet *et al.*, 1997) or excretion (Lu *et al.*, 2000) of the test substance.

A relatively novel concept regarding the role of PET in drug discovery is the contribution of this imaging technique to 'personalized medicine' (Eckelman *et al.*, 2008; Pither, 2003; Smith, 2005). Novel targeted anticancer drugs are often highly effective in a small subpopulation of patients, but the overall response rate is low. In order for these drugs to be effective, the target must be present during treatment. Radiolabeled peptides (or antibody fragments) and nuclear imaging techniques like PET can be applied to examine whether the target is expressed in the primary tumor or it metastases throughout the patient's body (Dijkers *et al.*, 2008; Van de Wiele *et al.*, 2008). PET scans are more informative than biopsy samples, since there is no risk of sampling errors and inaccessible (or unknown) lesions can be visualized. By providing proof of the presence of the target, PET can assist in the selection of the proper therapy for the individual patient.

The risk of application of costly therapies to non-responders (resulting in a dangerous loss of time and unwanted side effects) may be reduced and the success rate for the individual patient increased.

Because of the combination of positron-emitting radionuclides, specific radiopharmaceuticals and quantitative data analysis, PET offers unique possibilities to study drug pharmacokinetics and pharmacodynamics non-invasively in humans. Increasing cooperation between nuclear medicine specialists, basic scientists, and pharmaceutical companies will definitely lead to many future applications of PET in the drug discovery process.

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