

Applications of Positron Emission Tomography in Neuropsychiatric Pharmaceutical Drug Development

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Abstract: Positron Emission Tomography (PET) can be used to quantify proteins of interest in the brain, assess the function of these proteins, and quantify cerebral glucose metabolism and blood flow. Its value in neuropsychiatric pharmaceutical drug development is extensive, from the identification of relevant pathophysiology in disease states, to measurement of blood-brain barrier penetration and regional cerebral occupancy of a pharmaceutical agent, to predictions of treatment outcome from a specific pharmacologic intervention in a specific patient. In this paper, we briefly review some basics of brain imaging using PET, and describe its applications to the field of neuropsychiatric pharmaceutical development, including relevant examples from the existing literature. We conclude with a discussion of future developments that will make PET increasingly available and useful for such purposes.

INTRODUCTION

In 2004, the Food and Drug Administration (FDA) released an article titled: "Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products." In this document, they conclude: "Imaging technologies, such as molecular imaging tools in neuropsychiatric diseases or as measures of drug absorption and distribution, may provide powerful insights into the distribution, binding, and other biological effects of pharmaceuticals, but their predictive value needs further study and evaluation. New imaging technologies will ultimately contribute important biomarkers and surrogate endpoints, but how soon these new tools will be available for use will depend on the effort invested in developing them specifically for this purpose." In this article the FDA defines biomarkers as "quantitative measures of biological effects that provide informative links between mechanism of action and clinical effectiveness" and surrogate endpoint as "quantitative measures that can predict effectiveness." These definitions are in agreement with the Biomarker Definitions Working Group, who defined a biomarker as: "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic process, or pharmacologic responses to a therapeutic intervention." and surrogate endpoints as "A biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence" [1]. Currently, there are no neurologic or psychiatric drugs that have been approved based on their ability to alter either biomarkers or surrogate endpoints [2].

The purpose of this review is to describe how neuro-imaging using positron emission tomography (PET) is increasingly able to provide valid biomarkers and surrogate endpoints that will have relevance in the diagnosis and treatment of neuropsychiatric conditions. PET has already been used to identify neurochemical abnormalities related to stroke, Parkinson's Disease, amyotrophic lateral sclerosis (ALS), Alzheimer's Disease, central nervous system neoplasias, schizophrenia, multiple sclerosis (MS), depression, bipolar disorder, pain syndromes, and substance abuse, among others. PET can provide information about the pathophysiology of disorders, the blood brain barrier (BBB) permeability of a pharmaceutical, and the degree of occupancy of the target of interest by a pharmaceutical. It can identify subjects at risk, and predict treatment response. This information can and has been used to decide whether or not to

proceed with a new pharmaceutical or alter its dose in large-scale clinical trials reducing patient burden and costs.

BASICS OF POSITRON EMISSION TOMOGRAPHY

PET is an analytical nuclear medicine imaging technology that uses positron-labeled molecules at masses so low (typically less than 10 ug) that they are physiologically inert in order to image and measure the function of biological processes with minimum disturbance. The positron-labeled atoms within these molecules decay rapidly, releasing a positron, which subsequently collides with an electron, releasing two photons (511 KeV) in a process called positron annihilation. These photons are subsequently detected by the PET camera, and are used to reconstruct images [3]. This process can be used to quantify proteins of interest in the brain, cerebral glucose metabolism, or cerebral blood flow.

RADIOLIGAND DEVELOPMENT

There are several criteria that are required for a ligand to be effective for use in imaging with PET. A PET ligand is designed to bind specifically to a given molecular target in the brain. In order to do so, it must be selective for that molecular target (or at a minimum display selectivity for the target within relevant brain regions), and must bind the target with relatively high affinity. To cross the BBB, the ligand must be sufficiently lipophilic. It must be capable of being labeled with a radioactive isotope (most commonly ¹¹C or ¹⁸F), so that it can emit positrons as it decays, allowing detection by the PET camera. Radioligands are rapidly metabolized after they are injected, and it is important that the metabolites of a given radioligand be characterized, as quantification of this metabolism over time during the PET scan is required to obtain accurate estimates of binding. In addition, it is important that metabolites do not bind to the target, and ideally that they are polar, to minimize passage across the BBB. Of greatest importance, the radioligand must be safe when injected into humans.

The typical sequence of development of a PET radioligand is as follows: a clinical question or hypothesis will lead to the identification of a molecular target for which a radioligand is sought. The chemical structure of the candidate ligand should allow for a rapid labeling that does not destroy the pharmacological properties of the molecule. A candidate ligand that is not yet radioactively labeled will first be synthesized and assessed for affinity and selectivity for the target molecule using *in vitro* assays, followed by cellular assays. A ligand that passes these assays will then be radiolabeled. The stability of the radioligand over time will be assessed *in vitro*, as will its cellular uptake. MicroPET in rats or whole body scans in animals

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will be performed to measure the radioligand's biodistribution (its relative accumulation within different organs in the body). The time course of conversion of radioligand to metabolites can be measured from this PET scan, and tracer kinetic modeling can be performed to allow for full quantification. Based on these results, the sensitivity of the ligand to answer the original clinical question is assessed. *In vitro* binding is compared to *in vivo* binding. The specificity of the radioligand for its target is determined by blocking or chasing studies, in which a non-radioactive ("cold") ligand with known specificity for the target is administered to compete with the radioligand, and changes in resultant PET signal are assessed. Whole body dosimetry is then estimated in the non-human primates, and the toxicity of the radioligand at the relevant doses is assessed. A drug master file (DMF) is completed along with a pre-investigational new drug (IND) application, which are sent to the Food and Drug Administration (FDA). An institutional review board (IRB) application is submitted within a local academic institution for use of the radioligand in a human study, and the IND application is submitted formally to the FDA. Finally, a new drug application (NDA) is submitted to the FDA. This entire sequence leads to the exclusion of a vast number of possible radioligands at various points along the path, with a small fraction being suitable for and receiving approval for use in human populations.

IMAGE ANALYSIS

There are several ways in which to use PET data, from qualitative to fully quantitative. A qualitative approach is to evaluate an individual subject's PET scan through visual inspection alone. This is used, for example, when a radiologist evaluates a clinical PET scan of cerebral glucose metabolism that had been conducted on a patient with dementia. This procedure is now reimbursable via Medicare, and is the first cerebral PET study with such a reimbursable indication.

Various types of modeling are used to arrive at an accurate quantification of receptors in the brain using PET data. These models are required due to the complexity of the system: binding of radioligand to target is dynamic over time, and activity measured by the PET camera represents a combination of specifically-bound, non-specifically bound, and free (unbound) radioligand. These quantities, in turn, arrive at the brain through passage of the free component of peripheral arterial radioligand across the BBB. These "compartments" in which radioligands may reside at any point in time are interdependent, and rate constants may describe the passage of ligand from one compartment to another (Fig. 1). Quantification of ligand through kinetic modeling involves estimation of these rate constants. Other methods of quantification include equilibrium and graphical methods, all of which are reviewed elegantly in [4].

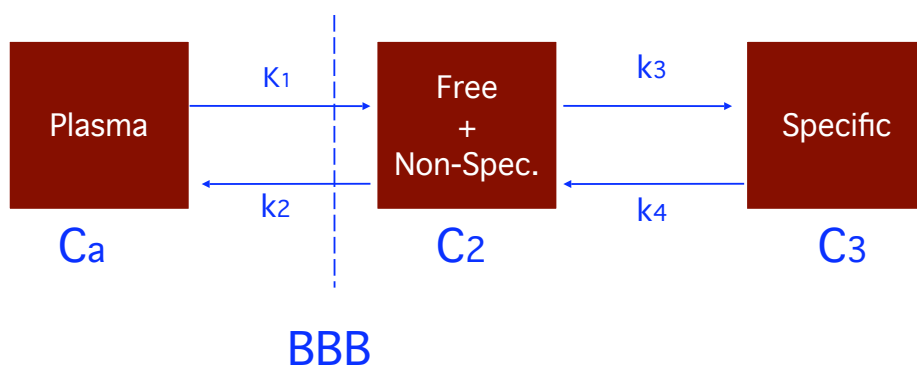


Fig. (1). Three-tissue compartment model. Following venous injection of radioligand into a subject, it distributes among three compartments: the plasma arterial compartment (C_a), the non-specifically bound and free compartment within the brain (C_2), and the specifically-bound compartment within the brain (C_3). The rate constants representing passage between these compartments are estimated as part of quantification of target.

The reference tissue method is a semi-quantitative approach. Anatomical regions of interest are identified on the PET scan (described in greater detail below), including a reference region. The outcome measure obtained using reference tissue methods, BP_{ND} , is termed semi-quantitative, as it requires the assumption that non-specific, or non-displaceable, binding of the radioligand is equivalent between the groups being compared [5]. A fully quantitative approach, which does not make this assumption, requires placement of a radial-artery catheter, from which blood samples are collected during the course of the PET scan. Radioactivity in these samples is quantified to obtain an arterial input function, representing the amount of ligand available to enter the brain over time. The arterial input function is incorporated with PET data into mathematical models to derive one of two possible outcome measures: if the free fraction of the radioligand in plasma (i.e. the portion that is not protein-bound), termed f_p , can be measured, then the optimal outcome measure, BP_F , can be estimated. If f_p cannot be measured due to the limitations of the radioligand, or is not measured by choice, then BP_P can be obtained, which requires the assumption that f_p is equivalent across subjects.

Four common outcome measures used in PET are defined here mathematically: total volume of distribution (V_T), binding potential (BP_F and BP_P), and specific-to-nonspecific equilibrium partition coefficient (BP_{ND}). The solution to the linear first-order differential equations that constitute the system of compartmental models typically applied in neuroreceptor PET studies is:

$$C(t) = \sum_{j=1}^m \phi_j e^{-\theta_j t} \otimes C_p(t) \quad (1)$$

where $C_p(t)$ is the concentration of the radioligand in the plasma (corrected for metabolism) over time and m is the number of tissue compartments in the model. For these models, V_T is defined as:

$$V_T = \sum_{j=1}^m \frac{\phi_j}{\theta_j} \quad (2)$$

The other outcome measures can be derived from V_T . Binding potential is defined as:

$$BP_F = \frac{V_T - V_{ND}}{f_p} \quad (3)$$

for a suitably chosen reference region, where f_p is the plasma free fraction and V_{ND} is the V_T of a reference region thought to have a very low density of the protein of interest. The other outcome

measures considered (also called “binding potential” in some references) are:

$$BP_P = f_P \times BP_F; \quad BP_{ND} = f_{ND} \times BP_F = BP_P / V_{ND} \quad (4)$$

where f_2 represents the free fraction of radioligand in the non-displaceable tissue compartment. Thus, both BP_P and BP_{ND} can be computed without measuring free fraction.

IMAGE PROCESSING

In addition to conducting a PET scan, rigorous quantitative analysis of PET images requires obtaining a T1-weighted MRI scan on the same patient, in order to identify anatomic regions of interest, as the spatial resolution of MRI is significantly higher than PET, allowing for more accurate determination of anatomic regions. The MRI image, and the regions of interest that have been identified on it, are then co-registered (brought into the same 3-dimensional space) to the PET scan, so the ROIs can be applied to the PET image. As PET scanning generates a series of images acquired over a period of as many as 120 minutes, the orientation of these images must be re-aligned to account for head movement by the subject during the scan. There are several types of motion correction, some based on automated alignment of images on the basis of shapes and contrasts in the images, others based on direct measurement of movement during the scan, which can then be inversely applied to images after the scan.

MODELING AND QUANTIFICATION

The relevant ROIs are then applied to motion-corrected PET images to extract a time-activity curve of radioactive counts detected by the PET camera within a ROI over time. The metabolite-corrected arterial input function and the time activity curves for ROIs as well as the reference region then serve as inputs for mathematical or graphical models which are used to estimate the outcome measures described above.

CLINICAL APPLICATIONS

There already exists a broad array of molecular targets for which there are suitable PET ligands. These include specific receptors and/or transporters for multiple neurotransmitters, such as dopamine, serotonin, norepinephrine, endocannabinoids, opioids, GABA, glutamate, and adenosine. Other molecular targets with existing PET ligands include fatty acids and amyloid. In addition, cerebral glucose metabolism can be assessed using an ^{18}F -labeled glucose radioligand (^{18}F fluorodeoxyglucose, or FDG) [6], and cerebral blood flow can be measured using radiolabeled water (H_2^{15}O) [7].

Performing PET with such ligands can be used to answer many questions that are relevant to drug development.

1. One can determine the number and distribution of a molecule that is being considered as a target for drug development, both in the brain and in the body as a whole.
2. The blood-brain barrier permeability of a candidate drug can be assessed, on the basis of displacement of radioligand by candidate drug.
3. Similarly, the occupancy of the target by the candidate drug can be measured. This is particularly relevant when there is a correlation between occupancy and clinical effects, or between occupancy and relevant side-effects. One example of this comes from the schizophrenia literature, in which there is some evidence that, in the case of typical antipsychotic medications whose primary effect is D2 antagonism, clinical response is observed when medications achieve at least 65% occupancy of striatal D2 receptors, and extrapyramidal motor side-effects are observed more commonly when striatal D2 occupancy is above 75-80% [8]. The generalizability of this finding is limited by the

fact that atypical antipsychotics achieve significant clinical effects with lower, or more transient, striatal D2 occupancy [9].

4. PET can be used to elucidate the pathophysiology of a disease of interest, by comparing the density and distribution of a relevant molecule between patients with the disease and an unaffected comparison group. An example of this is a recent study of the serotonin 1A (5HT_{1A}) receptor. Our group first established the dosimetry and biodistribution [10], modeling parameters [11], choice of reference region [12], and characteristics in a healthy controls [13], of a 5HT_{1A} radioligand, ^{11}C -WAY100,635, which is a 5HT_{1A} receptor antagonist. The dosimetry and biodistribution study identified the bladder as a major site of accumulation of radioligand, at odds with animal dosimetry studies, and was useful in determining doses of radioligand that are safe for use in humans (Fig. 2). We next compared 5HT_{1A} binding potential (BP_F) using the ^{11}C -WAY100,635 ligand between a group of patients with major depressive disorder (MDD) in the midst of a major depressive episode and a group of healthy comparison subjects. While there was no difference in BP_F between patients with MDD and the comparison group when the MDD group was considered as a whole, subgroup analysis revealed a significant effect of prior antidepressant use, such that antidepressant-naïve patients demonstrated significantly higher 5HT_{1A} BP_F than antidepressant-exposed patients or healthy comparison subjects [14]. This finding provides evidence for an elevation of 5HT_{1A} receptors in MDD that may be normalized by treatment interventions. An example image of WAY binding that was generated using parametric voxel-wise modeling (as opposed to modeling at the level of an entire region of interest) is found in (Fig. 3).
5. Another relevant function of PET scanning, which has also been demonstrated with the 5HT_{1A} receptor, is to identify different populations of receptors on the basis of their affinity-state. In the case of the 5HT_{1A} receptor, this can be achieved because 5HT_{1A} antagonists (such as WAY, from which the ^{11}C -WAY100,635 ligand is derived) bind to both low- and high-affinity receptors, whereas 5HT_{1A} agonists bind preferentially to high-affinity receptors, which are differentially located and serve different functions in the brain [15]. This agonist ligand may also be used in the future to assess intra-synaptic levels of endogenous serotonin, and to assess desensitization of pre-synaptic 5HT_{1A} receptors [16].
6. PET can be used to predict which patients are most likely to respond to treatment for a specific condition. We assessed baseline 5-HT_{1A} BP_F as a predictor of one-year remission from major depressive disorder (MDD) in a group of patients who received naturalistic antidepressant treatment, in which a majority of patients (73%) received selective serotonin reuptake inhibitors (SSRIs) [17]. Remission was defined by $\geq 50\%$ reduction in 24-item Hamilton Rating Scale for Depression (HAM-D) score and a final score of ≤ 10 . Eventual non-remitters had higher 5-HT_{1A} BP_F across all brain regions at baseline than remitters ($p=0.025$). Based on this finding, we are currently conducting a prospective study predicting treatment response in MDD with standardized treatment with the SSRI escitalopram.
7. PET can also be used to predict which patients are likely to develop a given clinical condition, which could thereby lead to pharmacological interventions aimed at prevention or modification of disease progression. A very promising example of this is the case of Alzheimer's Disease. A definitive diagnosis of Alzheimer's Disease has up until now been only possible by histopathological study of post-mortem brain tissue, on the basis of the presence of neuritic plaques containing amyloid-beta ($A\beta$) [18]. A radioligand has recently been developed that specifically binds $A\beta$ in the brain, ^{11}C]PIB, which has been found in several studies to differentiate groups of patients with Alzheimer's Disease from healthy controls [19]. There remains significant

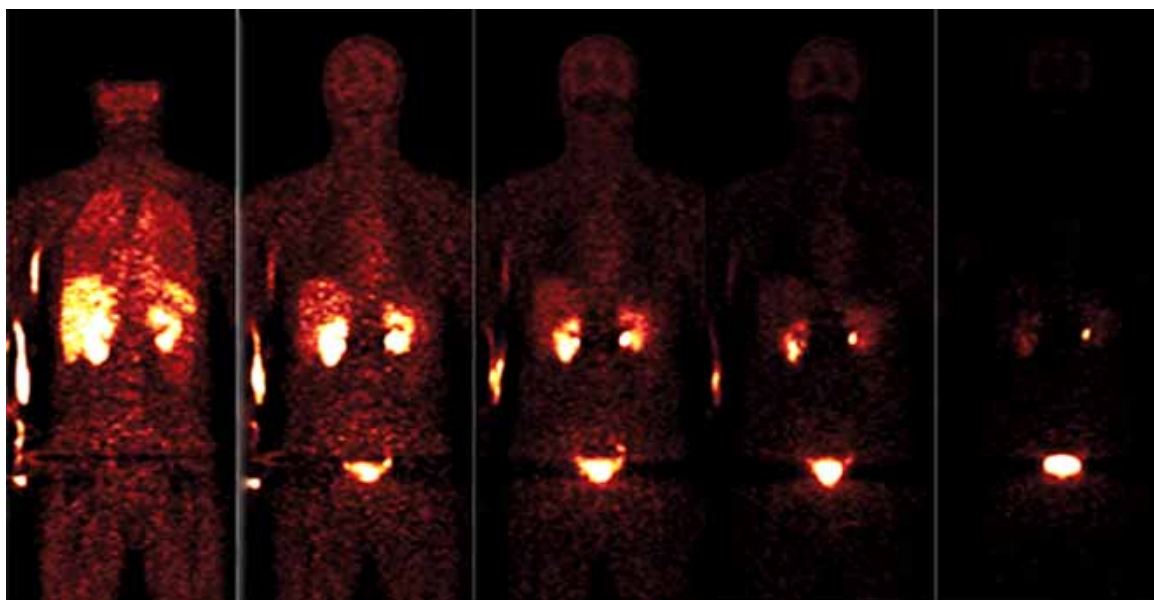


Fig. (2). Human biodistribution study with the 5HT_{1A} receptor ligand ¹¹C-WAY100,635, demonstrating significant uptake in the bladder. Coronal slices taken as a function of increasing time.

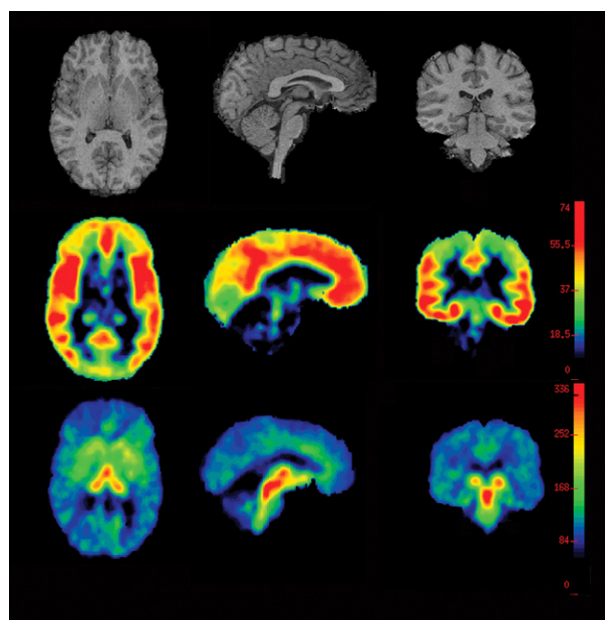


Fig. (3). Quantification of the serotonin 1A (5HT_{1A}) receptor and serotonin transporter (SERT) using PET. Top: structural T1-weighted magnetic resonance imaging of a subject's brain in axial (left), sagittal (middle), and coronal (right), views. Middle: 5HT_{1A} receptor BP_F using parametric voxel-wise kinetic modeling with the ¹¹C-WAY100,635 ligand, co-registered to the above MRI image. Bottom: SERT receptor BP_F using parametric voxel-wise kinetic modeling with the ¹¹C-DASB ligand, co-registered to the above MRI image.

overlap between groups in such studies, however. New techniques, including voxel and cluster-based methods that do not require assumptions regarding the functional anatomic boundaries of regions of interest, may soon allow clinicians to make valid predictions about the likelihood of at-risk individuals to develop Alzheimer's Disease, to track disease progression in a quantitative measure on the basis of amyloid burden, and to assess the effects of therapeutic interventions on disease progression.

Using some or all of the information described above, PET can also be used to make go-no go decisions, and to reduce the costs for trials.

There are some challenges that remain in the implementation of PET for identifying biomarkers and surrogate endpoints that are relevant for the purpose of drug development. One is that psychiatric illnesses are in general slowly-evolving, making pathophysiological changes more challenging to track over time. An additional problem is that of standardization of methodology across sites where these technologies are used, as both the equipment used (including PET cameras) and the modes of acquiring and analyzing data can differ significantly in different sites. The stability over time of the instruments used for PET scanning is not a given, and quality assurance must be performed to assess this. There are opportunities for operator error in many steps along the pathway. The many sources of variance, including noise in estimations when quantifying

molecules of interest, as well as inter-subject variability within a given condition, mean that sample sizes needed to detect differences between populations may be large in some cases. This may limit the applicability of PET findings for individual patients, although advances in image processing, kinetic modeling, and statistical analysis will likely make this more and more feasible in the near future.

THE FUTURE

There are several methodological advances which our group and others are developing currently that will make PET imaging more relevant, inexpensive, and available for pharmaceutical development as well as use in clinical practice:

1. We are working on creating PET radioligands that will be stable enough to be shipped remotely to other sites. This can be accomplished by incorporating ^{18}F as opposed to ^{11}C into radioligands, due to its significantly longer half-life.
2. We are developing methods that allow automated region of interest (ROI) determination, which will lead to remarkable savings of time in the analysis of PET data compared to manually-drawn ROIs, and significantly higher accuracy than can be achieved by warping individual brains, which differ in regional anatomy, into a standardized space [20]. An example of such automated ROI determination can be found at: <http://www.binarybottle.com/mindboggle.php>
3. We are working to create increasingly automated image processing streams, in order to increase ease of implementation by different user groups, increase reproducibility, and increase efficiency. An example of a semi-automated image processing stream, used for analysis of both PET and functional MRI data, is FSL, produced at the Oxford University, freely available at <http://www.fmrib.ox.ac.uk/fsl/> [21].
4. We are developing methods in which an arterial input function could be estimated from the PET data itself, obviating the need for radial artery catheterization. One principle of such methods involves identifying a blood vessel compartment within or outside the brain within which activity is quantified over time; such techniques are sometimes called image-derived input functions for this reason. They have already been implemented by other groups to quantify cerebral glucose metabolism using FDG [22].

5. Finally, we are developing novel voxel-based regression methods to allow us to assign the probability of illness or of treatment response in a multifactorial setting, incorporating relevant predictors including clinical variables and different imaging modalities [23].

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