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Radiopharmaceutical pharmacokinetics in animals: critical considerations

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Since the advent of single photon emission computerized tomography (SPECT) and positron emission tomography (PET) various chemical ligands have been labeled with radionuclides and evaluated as tracer compounds in animal models to ascertain their suitability as potential radiopharmaceuticals for humans. In the absence of a defined algorithm to predict the diagnostic efficacy of a radiopharmaceutical, any new radioligand has to undergo preclinical evaluation even if it has excellent *in vitro* properties. Until now few studies have produced pharmacokinetic data that could be translated from animal models directly to humans. The purpose of this review is to highlight some critical aspects to consider during the development and validation phase of a new radiopharmaceutical. Interspecies differences and the absence of knowledge of physiological mechanism can become challenging drawbacks for obtaining a successful radiopharmaceutical. In this context, the influence of ABC transporters in neuroimaging, the effect of plasma protein binding and the consequence of anesthesia with reference to interspecies differences will be discussed with illustrative examples.

Key words: **Radiopharmaceuticals - Pharmacokinetics - Animals.**

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are currently the most powerful imaging techniques for non-invasive assessment and quantification of biochemical pathways, molecular interactions, cancerous lesions, and brain receptors using a radioactively labelled probe or drug. Over the past two decades the application of imaging to evaluate new drugs and their effects in living organisms has provided many critical insights about the pharmacokinetic and pharmacodynamic properties of several classes of drugs (*e.g.*, neuroleptics, antineoplastics, antimicrobials etc.).

The complete sequencing of the human genome and the advent of proteomics and genomics have led to the discovery of novel molecular targets and to the development of new or better animal models for testing drugs. In parallel, improvements in PET or SPECT instrumentation and image analysis together with the availability of suitable radiotracers have made molecular imaging a rapidly expanding field of preclinical and clinical investigations. As summarized in Figure 1, the radiopharmaceutical development process for either PET or SPECT is complex and multidisciplinary. Although many candidate radiotracers have been developed for imaging, only a small number have found application as radiopharmaceuticals for *in vivo* imaging in humans. Although the potential radioligand may possess the desired *in vitro* characteristics, other factors such as *in vivo* pharmacodynamics and pharmacokinetics contribute to a greater extent to its failure. *In vivo* pharmacokinetics is generally evaluated

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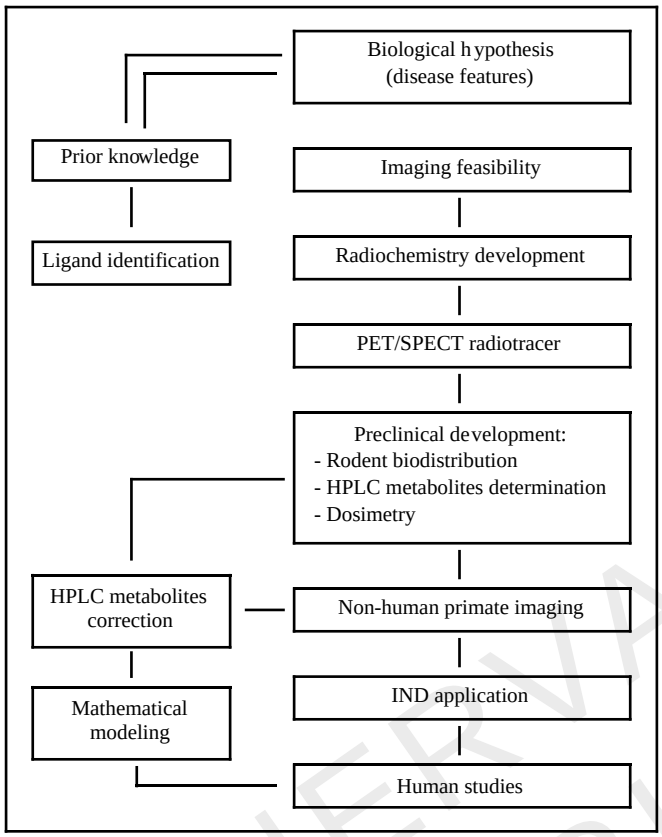

Figure 1.—Overview of radiopharmaceutical development process.

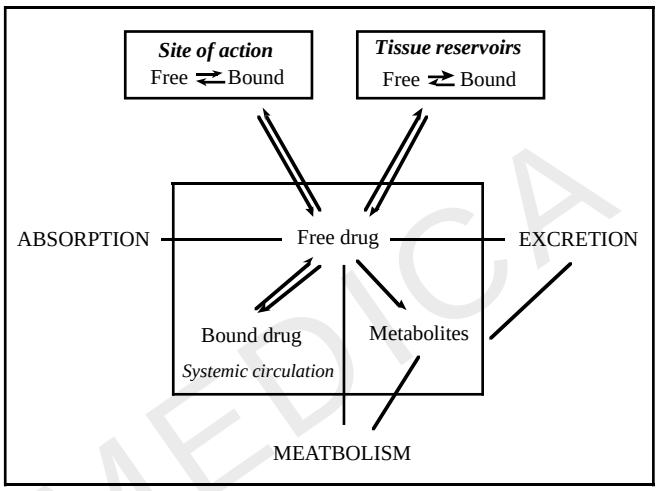

Figure 2.—The effect of reversible drug-protein binding on drug distribution and elimination in biological systems.

to estimate the absorption, distribution, metabolism and excretion (commonly referred to as ADME) of an administered radiopharmaceutical, the rate at which its action begins and the duration of its imaging effect.[1] An ideal radiotracer candidate should possess both high target affinity as well as good pharmacokinetics properties. For example, without the latter, the effect of a radiotracer may be so short-lived that its imaging benefits may be minimal. In the last twenty years, the importance of early pharmacokinetic screening has become well-recognized in the radiopharmaceutical discovery and development process (Figure 1). Figure 2 summarizes common issues in drug discovery that arise due to undesirable ADME properties, and some of the assays, typically used to evaluate these properties and to expedite compound selection in lead optimization. This review describes with illustrative examples some critical issues to be considered during the initial pharmacokinetic evaluation of a new radiopharmaceutical.

Implications of ATP-binding cassette transporters for neuroimaging

The blood-brain barrier (BBB) limits the accumulation of several drugs in the central nervous system (CNS) and represents a great challenge to the delivery of therapeutic or diagnostic compounds in the brain. In 1976, a class of transporters characterized as energy-dependent, membrane-bound proteins was discovered to be expressed in several tissues, including the BBB.[2] These efflux transporters were found to play a gatekeeper role, specifically limiting drug penetration at the luminal side (blood side) of the brain capillary endothelial cells (thereby preventing the diffusion to the CNS) as well as mediating the secretion of drugs at the abluminal side (bile/urine side) of the endothelial cells in the liver and kidney (thereby limiting overall reabsorption). Although the BBB allows the ingress of lipophilic uncharged molecules with low molecular weight by passive diffusion, many lipophilic drugs show negligible brain uptake.[3] The efflux transporter P-glycoprotein (P-gp; ABCB1) a 170 kD plasma membrane protein product of the MDR1 gene, is considered a major biochemical component of the blood-brain drug permeability barrier and

TABLE I.—*Potential small molecule ADME issues and assays frequently employed to address them.*

Absorption	Distribution	Excretion/metabolism
Low membrane permeability	*High plasma protein binding*	*High gut metabolism*
— In vitro assays (e.g. Caco-2 or MDCK cells)	— Ultrafiltration/Ultracentrifugation — Equilibrium dialysis	— Caco-2 stability — Microsome stability
High P-gp efflux	*Poor BBB penetration*	*High hepatic metabolism*
— Caco-2 P-gp screening — Inhibition of P-gp activity	— *In situ* perfusion studies — Brain/CSF collection — *In vivo* imaging	— Microsome stability — S9 stability — Hepatocyte/tissue slices — Metabolites identification
Poor physicochemical features		*Enzyme induction or inhibition*
— Solubility — Log P — Log D — pKa — pH stability		— Promoter/reporter gene/cell based assays — P450 mRNA, protein expression or activity measurements — Enzyme specific inhibition assays
		Poor biological fluids stability
		— Plasma stability
		High transporter-mediated excretion
		— Renal/biliary transporter activity

belongs to the ATP-binding cassette (ABC) superfamily of transmembrane proteins. Its role is to protect the brain from environmental toxins and toxic substances under physiological conditions and to inhibit the accumulation of pharmacological compounds in the diseased brain, thus reducing drug efficacy.[4] In therapy the effect of being a substrate of ABC transporters can be overcome by increasing the dose given which in turn may reduce the therapeutic window. For a radiotracer, this is not a solution. On the other hand, this feature has been used to image the presence of the P-gp in tumor tissues.[5]

A critical aspect of ABC transporters is that their expression and function show strong variability between animal species and strains and also can change in response to pathophysiological conditions.[4, 6] This complexity poses impediments in radiopharmaceutical development for neuroimaging, especially because a class of transporters may be expressed by one species but not by another. Furthermore, the brain pathology of an animal model can invariably alter radiotracer biodistribution and has a crucial influence on its accumulation in the CNS. Consequently, pharmacokinetic data from mice might not translate to rodents and accordingly cannot be necessarily adapted to humans. Considering the important effects of ABC transporters on radiopharmaceutical biodistribution, inhibition of ABC transporters is a challenging solution for abolishing the efflux of an investigational radiotracer. The recent discovery that cyclosporine A and verapamil possess the ability to block ABC transporter activity, has paved the way to study more potent and selective inhibitors of ABC transporters, possibly lacking in side-effects and interactions with other therapeutics.[7] Current research in non-human primates or rodents has shown the feasibility of imaging to evaluate the function of ABC transporters with radiotracers, such as [^{68}Ga]-complexes or [^{18}F]-placlitaxel or to test the ability of selective transporter deactivators to increase brain concentration of [^{11}C]-verapamil.[4] Besides [^{11}C]-verapamil, the most frequently used PET radioligand for specifically evaluating P-gp function, other radiotracers such as [^{11}C]-carazolol, [^{18}F]-fluorocarazolol, [^{11}C]-carvedilol, [^{11}C]-GR218231, and [^{11}C]-loperamide have been recently evaluated as P-gp substrates. Some of these have been shown to be P-gp substrates in rodents but not in primates, demonstrating the influence of species differences in P-gp function. A few examples are the 5HT1A receptor antagonists [^{11}C]-RWAY and [^{18}F]-MPPF, the 5HT2A receptor antagonist [^{18}F]-altanserin, and the NK1 receptor antagonist GR205171. All of them possess affinity for P-gp in rodents but not in primate or human brains as confirmed by their different levels of accumula-

tion in the brain of these species.[6] Despite the high degree of homology in the amino acid content of P-gp in different species, these findings point out that differences between species are present and are possibly due to dissimilarities in P-gp function, expression, transport, capacity or substrate affinity. Also other factors not related to P-gp may be behind this different behavior in different species, such as differences in the biological structure of the BBB, *e.g.* components of the endothelial cell tight junctions or expression of other efflux transporters. Another possible explanation of the reduced intrabrain distribution in rodents compared to humans is differences in the ratio of lipophilic/hydrophilic content of the brain tissue, and in the degree of plasma protein binding, distribution to other tissues, metabolism, and excretion from plasma.[6]

Species differences should be kept in mind when extrapolating data obtained in animal models to humans. Radiotracers found to be substrates of ABC transporters in rodents are likely to also behave as substrates in higher species, but in humans enough BBB permeability may be retained to allow radiotracer binding at intracerebral targets.

Plasma protein binding

After a drug is administered into plasma, its molecules are distributed throughout the body by systemic circulation and can reach the target sites (receptors), other non-receptor tissues such as eliminating organs (liver and kidneys) and non-eliminating tissues (brain, heart, muscle, skin, fat tissue etc; Figure 3). Drug binding to plasma proteins greatly influences the drug distribution because it hinders partitioning across the capillary membranes into tissues. Plasma binding determination is essential for characterizing pharmacokinetic profiles and estimating the drug overall pharmacological activity. The free fraction of a drug in plasma is generally considered the principle parameter of drugs availability for distribution to tissues, entry into cells, interactions with receptor sites and elimination by excretion or metabolism (Figure 3). Drug transport or diffusion into tissues is governed by the free drug concentration gradient between plasma and tissue, until equilibrium is reached.[8, 9] A substantial portion of the drug may be bound to proteins in plasma. The most important plasma proteins are serum albumin (SA), alpha1-acid glycoprotein (AGP) and lipoproteins which have structurally selective binding sites for drugs. SA is the most abundant plasma protein and is composed of a single polypeptide chain of approximately 590 amino acids with a molecular mass of 66.5 kDa.[10, 11] Its main function is to regulate blood osmotic pressure. Furthermore, it serves as a transport protein for several endogenous compounds, such as unesterified fatty acids and bilirubin, and it is also capable of binding a broad spectrum of therapeutic agents. Drug binding to SA can result in prolonged *in vivo* half-life. Thus, the binding properties of drugs to human SA are one of the most important factors determining their pharmacokinetics.[10, 11]

Although structural and functional homologies of SA exist among species due to small changes in the amino acid sequences between human and animal species, it has been shown that variable drug binding affinity to nonhuman serum albumins are not predictable based on drug affinities for human SA. These differences can be particularly important in radiopharmaceutical research and development and may impact translation of pharmacokinetic findings between animal models and humans.[8, 12] N-isopropyl-p-[^{123}I] iodoamphetamine is one illustrative example in this context. In fact this tracer was shown to bind differently to plasma proteins in three different species, specifically rat, monkey and human.[13] Moreover, significant variations in the level of binding to serum albumin of five different species including humans, have been shown *in vitro* for copper(II) bis(thiosemicarbazone) radiopharmaceuticals.[14]

Considering that the volume of distribution and the concentration of a drug at the receptor site are strictly related to the fraction of unbound drug, a full characterization of the binding mechanism to proteins such as SA (and maybe AGP) has become essential for understanding the pharmacological profile of any radiopharmaceutical, to avoid misinterpretation of imaging results into the tissues of interest. Tight binding or slow dissociation rate from non specific proteins or tissues determine a reduction of the free fraction of the tracer available to diffuse (*i.e.* across the BBB) and interact with its specific targets (i.e. receptors, enzymes etc.). This produces high background and low signal-to-noise ratio. Therefore, understanding the impact of non-specific interactions on *in vivo* binding of a radiotracer to its specific targets is essential, since significant species-to-species variations may impact the reliability of animal models in predicting

radiopharmaceutical efficacy and performance in humans.

Influence of anesthesia on *in vivo* pharmacokinetics

Contrary to human studies, PET or SPECT in small animals generally require anesthesia to prevent animal movement during the scan and therefore reduce imaging artifacts. Lately it has been recognized that anesthesia influences the *in vivo* pharmacokinetics of injected radiotracers in living animals by altering cardiovascular, respiratory, and central nervous functions and ultimately inducing pulmonary atelectasis, hypoxia, hypothermia, acidosis and hepatic toxicity.[15]

The duration of sedation and the anesthetic protocol can induce several effects which are hard to predict *a priori*, including density, availability, binding of target proteins for structurally unrelated radiotracers for a specific binding site. Changes of the relative blood perfusion and the blood volume of the studied organs, and consequent changes in compound elimination velocity have provided contradictory results among different classes of anesthetic agents.[16] Also tracer uptake, kinetics and metabolism may be altered *in vivo* by use of anesthesia, thus complicating data interpretation and modeling in sedated animals. Anesthetic agents should be easily administered and effective for the whole duration of the scan. Therefore, prolonged inhalant anesthesia (*e.g.* isoflurane) is the most consistent and convenient regimen in small animal imaging and is relatively safe for spontaneously breathing animals when delivered with a precision vaporizer but it can interfere with research studies.[15] In fact, aside from global effects like hypothermia, isoflurane has been shown to augment cerebral blood flow in rats and mice, to increase the extracellular dopamine concentration and to inhibit the high-affinity dopamine D2 receptor state. As a result, isoflurane inhalation was proven to interfere with [^{123}I]-IBZM brain uptake and clearance in mice.[17] However, in a PET study in monkeys, the non-displaceable binding potential of [^{11}C]-raclopride was not affected by the administration of isoflurane/N_2O.[18] These two examples point out how anesthesia can influence the pharmacokinetics/pharmacodynamics of radiopharmaceuticals.

In mice, immunomodulatory effects have been documented for up to 9 days following three 40-min weekly exposures to sevoflurane.[19] Consequently, in longitudinal studies, the time period between imaging sessions should be carefully considered and the effects of anesthesia fully understood. Besides inhalant anesthesia other commonly used drugs for sedation are pentobarbital, propofol and ketamine/xylazine. Pentobarbital has been shown to decrease the elimination velocity and the distribution of [^{123}I]-2-iodo-l-phenylalanine toward the peripheral compartment, leading to less tumor uptake and higher blood pool and kidney activities.[20] The use of either isoflurane or sodium pentobarbital increased significantly the binding of N-[^{18}F]fluoroethyl-piperidinyl benzilate ([^{18}F]FEPB), a high affinity antagonist for the muscarinic cholinergic receptor, in receptor-rich brain regions relative to awake controls. Blocking of the enzyme acetylcholinesterase with phenserine did not produce further increases in radioligand binding, in contrast to the marked increases (>70%) previously observed in awake animals after drug treatment.[16] These results indicate how anesthesia can produce significant changes in baseline biochemical values which, in turn, can obscure significant effects of pharmacological challenges. In a recent study, the impact of anesthesia on [^{18}F]-FDG distribution was determined in normal mice under the following conditions: no anesthesia, ketamine and xylazine (Ke/Xy), 0.5% isoflurane, 1% isoflurane and 2% isoflurane. Higher blood glucose levels and marked muscle uptake of [^{18}F]-FDG was found in the Ke/Xy anesthesia group compared with the other ones. Also, isoflurane increased [^{18}F]-FDG activity in the lung, heart, kidney and intestine in a dose-dependant manner. These findings are consistent with the properties of isoflurane, which causes a decrease in blood pressure, respiration and glomerular filtration rate during maintenance of anesthesia in relation to the depth of anesthesia.[21]

An optimal anesthetic regimen for long-term scanning (>30 min) should consist of a continuous monitoring of the adequacy of anesthesia depth by checking respiratory frequency, fluid supplementation in order to ensure homeostasis of fluids, of electrolyte balance and blood glucose levels, and of appropriate warmth to avoid hypothermia. Precise monitoring of these variables during anesthesia is crucial for the maintenance of physiological and hemodynamic stability within and across imaging studies and to ensure high intra- and interstudy reproducibility of data.

Strain, gender and age differences in response to anesthetics, such as the sleep time of anesthesia, stress, immunity, tumorigenesis and pharmacokinetics, also have been documented.[22] The effects of anesthesia should always be considered when examining pharmacological or behavioral perturbations of normal neurochemistry. The use of genetically engineered rodents should be evaluated carefully due to differences in susceptibility to anesthesia-associated morbidity and mortality.[15]

Conclusions

In conclusion *in vivo* preclinical testing of new radiopharmaceuticals is a repetitive process that requires luck and serendipity. The influence of anesthesia, plasma protein binding and P-gp are just a few illustrative examples of critical issues that need to be taken into consideration during the evaluation of a novel ligand in animal models but many more exist (*e.g.* metabolic transformations, mechanisms of renal and hepatic excretion, efflux transporters etc.) and keep the bridge from animal to human studies formidable and complex. This review even if far from exhaustive is intended to encourage scientists to exercise caution in their interpretation of results and to avoid the temptation to report only experimental successes. In fact, sometimes a negative result can turn into a positive finding if it is read and understood correctly.

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