Neuropharmacology and drug development

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Positron emission tomography (PET) and allied non-invasive imaging techniques are being increasingly embraced by the pharmaceutical industry. These imaging modalities allow the assessment of novel drug action in man at a very early stage of the drug's discovery and development process; in turn, this enables earlier decision making about the developmental potential of novel and potential therapeutics. The in vivo characterisation of novel molecular targets and disease mechanisms in man is intimately connected with future developments in the diagnosis, management and treatment of human disease. The utility of non-invasive imaging modalities within the pharmaceutical industry is discussed with particular reference to the use of PET in drug discovery and development in the 21st century.

Traditionally, the discovery and development of new drugs has been performed with a heavy emphasis on in vitro techniques to select promising lead candidates which are subsequently tested in living animals prior to human administration. Because in vitro systems reflect only part of the complexity of living systems and that in vivo animal models of human disease are often only an approximation of human pathology, there is growing realisation within the pharmaceutical industry that a robust understanding of drug–receptor/ enzyme interactions in living man at an early stage in this process will be a major driving force in further enhancing the effective, efficient and timely discovery and development of novel therapeutics. In order to address this crucial issue, the development of new paradigms for assessing drug action in man at an early stage in the drug discovery process are required – in effect making man the test animal in question. Over recent years, there has been a growing use of human medical imaging within the pharmaceutical industry to assess pathology, disease processes and drug action, in relation to the selection and evaluation of novel drug candidates from an early stage of development. These imaging modalities include PET, MRI, CT, ultrasound, EEG, SPECT and thermography to name a few. Although the techniques provide information about different aspects of living systems, the combined use of a number of these is likely to provide more information than any single modality alone, and can be seen as complementary tools in the arsenal of platform technologies available for increasing our understanding of drug action in...
man. For the purpose of this chapter, emphasis will be placed on the use of PET as an aid to drug discovery and development.

**Why PET?**

PET is being used increasingly by the pharmaceutical industry, for a number of reasons.

*Biogenic radionuclides*

The radionuclides commonly used in PET include isotopes of the biologically ubiquitous elements: carbon-11, nitrogen-13, and oxygen-15. As most drugs and endogenous compounds are made up of carbon, nitrogen and oxygen, in principle it is possible, to label all drugs with a positron emitter which are autologous with the parent compound. Although fluorine is not a common constituent of organic molecules, a positron emitting isotope of this element, fluorine-18, can sometimes be incorporated into a molecule of interest to produce an organo-fluorine analogue of the parent molecule without appreciably effecting its pharmacological and physiochemical properties.

*Short half-lives*

The radioactive half-lives of carbon-11, nitrogen-13, oxygen-15 and fluorine-18 are 20, 10, 2 and 110 min, respectively. These short half-lives endow a number of advantages to their use as tracers to probe biological processes *in vivo* using PET. Repeat studies in the same subject within the same day are made possible. Thus baseline and drug perturbed states can be measured rapidly, eliminating intersubject variability. The short half-lives also present a number of challenges. These include the need for rapid incorporation of the label into the molecule of interest, purification, formulation and sterilisation in a time scale compatible with the half-life of the relevant radionuclide. This also requires the siting of a cyclotron and radiochemistry laboratory in close proximity to the PET scanner.

*Tracer technique*

Positron emitting tracers can be produced with high specific activities (greater than 1 Ci/µmol). This means that the amount of drug administered to a subject is very low (typically less than 10 nmol) and at a sub-
pharmacological dose. Even highly potent or toxic compounds can be labelled and administered to living subjects without the manifestation of pharmacological and/or toxicological effects. For example, the potent \(\mu\)-opiate agonist carfentenil, used in small amounts to anaesthetise large animals, has been successfully used as a PET tracer for opiate receptors in living man without the manifestation of pharmacological effects\(^3\). The unique sensitivity of the PET technique allows tissue kinetics and the regional distribution of PET-labelled drug molecules to be assessed at very low doses. The implication of this is that reduced toxicology packages may be applied to novel drug candidates so that they may be assessed with PET in man in tracer doses at a very early stage of the drug discovery process, months or years ahead of what might be anticipated in a traditional drug development programme. Such an early evaluation of drug candidates in man may make a significant impact on the efficiency of drug development and associated socio-economic benefits.

**Perspectives on novel tracer and radioligand development**

Despite the challenges imposed by using the short lived positron emitting isotopes, a wide variety, of selective and specific radiotracers have been developed, including receptor ligands, enzyme substrates and inhibitors, amino acids and markers of blood flow, blood volume, oxygen utilisation\(^2,4\). Some of these tracers, in particular receptor ligands, have been used to assess normo- and pathophysiology and drug action in man. Drugs being developed at an early stage in the drug discovery pipeline often act at totally novel molecular sites and, as such, usually there are no existing radioligands acting at these receptors. Clearly, if this technique is to be used to its greatest potential within the pharmaceutical industry, PET tracers and ligands need to be developed in conjunction with the chemistry programmes around these molecular targets. For each molecular target for which drugs are being developed, hundreds or even thousands of molecules with different physicochemical and pharmacological properties are synthesised and tested before a single drug candidate is progressed. It is recognised that the properties of a drug are not always synonymous with the desired properties of a PET ligand. The careful screening of the compound libraries provides a rich source of alternative compounds that may yield valuable PET ligands. In conjunction with considerable expertise (e.g. medicinal chemistry, neuropharmacology, drug metabolism and pharmacokinetics, toxicology and clinical pharmacology) the pharmaceutical industry is well placed to facilitate focused and systematic development of novel PET radioligand candidates. Because of sensitivities around novel chemical structures and intellectual property rights, there is clearly a
need for pharmaceutical companies to develop a critical mass of imaging expertise and secure links to academic PET centres in order to assure the most beneficial use of these precious resources. Key alliances between industry and academic centres are crucial for the implementation of these novel probes in answering questions related to pathophysiology and the assessment of drug action in man.

**The application of PET to drug development in the pharmaceutical industry**

There are number of ways in which the PET technique can be applied to drug discovery and development. The following sections briefly describe some of these.

**Biodistribution studies**
*(radiotracer is a positron emitting autologue of the drug candidate)*

PET biodistribution studies (sometimes termed ‘microdosing’) use radiotracers which are positron-emitting autologues of the drug candidate itself. The regional distribution, tissue and plasma kinetics of drug candidates is often well understood in preclinical species, prior to the administration of the drug to man. However, as eluded to earlier, significant differences in behaviour are often seen in the end-target species, man. In order to examine whether a novel drug molecule enters the therapeutic organ of interest, study of the tissue kinetics and distribution of the drug candidates in man is required. This is particularly attractive where the drug acts at organs where it is difficult to perform tissue biopsies to assess drug concentrations. A typical example of this type of study is in assessing the brain entry of CNS-active compounds in man. Not surprisingly, significant differences in brain concentrations of a drug across species have been observed. Labelling the drug with a positron-emitting radionuclide and performing a non-invasive and quantitative PET biodistribution study early on in the life-cycle of a drug may contribute valuable information about the development potential of drugs in man, and maximise the resource and time commitment to various drug development programmes.

Biodistribution studies should be designed and executed carefully for a number of reasons.

**Metabolism**

At tracer doses, the break down of the parent molecule may become first order with respect to the concentration of the degradative enzymes. Thus relative plasma concentrations of parent and metabolite may not
be identical at a tracer or pharmacological dose. Therefore, character-
isation of the fate of the labelled compound from carrier-free to
appropriate carrier-added doses is required. This is often performed in
preclinical work-up studies in advance of a human investigation, but
should eventually be tested in living man at a point where sufficient
toxicological information about the test drug is available so that non-
tracer doses of the drug can be administered.

**Position of labelling**
The drug’s site of labelling needs to be carefully considered, so that the
identity of tissue-bound radioactivity is clearly understood. In the case of
CNS compounds, labelling the molecule in a position whereby only
labelled hydrophilic metabolites are produced precludes the contamination
of the CNS signal from labelled metabolites. Again, the study of the fate
of the radiolabel in preclinical species needs to be carefully examined, prior
to the human study, in order to validate this approach.

**Quantitation**
In addition to having a clear understanding of the dose linearity and
metabolic fate of the labelled drug, careful attention should be given to
the study design when performing a biodistribution investigation. For
compounds that are poorly CNS penetrant, the vascular component of
the CNS signal may significantly contribute to the perceived tissue
concentration of the drug. In such cases, accurate blood volume
correction is essential. Scattered radiation can also significantly
contribute to the observed radioactivity concentration in the tissue of
interest. Biodistribution studies should be designed, where possible, so
that the potential impact of scattered radiation to the tissue signal can
be assessed and quantified. Co-registration of the PET scans to
anatomical imaging modalities is essential so that regions of interest can
be accurately defined from the PET-scan data. This is particularly
helpful where the drug concentration in a specific tissue is low or where
anatomical landmarks are poorly represented within the PET data-set.

**Optimising drug-dosing regimen**
Traditionally, the optimisation of a drug-dosing regimen in man has been
performed using a number of initial assumptions based on relationships of
drug concentrations in plasma to the target-tissue concentrations from
animal data. These initial assumptions are subsequently refined in later
phase clinical trials in target populations where pharmacodynamic end-
points can be used to fine-tune an optimal dosing regimen. However, there
are a number of caveats to these assumptions.
Drugs often exhibit a disconnect in the plasma and tissue kinetics, e.g. the presumption that the absence of drug due to its clearance from plasma reflects a corresponding absence of drug in the tissue of interest is incorrect. Clearly, to achieve steady-state tissue drug concentrations and to avoid unnecessary adverse events or toxicology, the characterisation of the drug half-life of enzyme/receptor occupancy is highly desirable.

In certain diseases, the pharmacodynamic end-points used to fine-tune a drug-dosing regimen in man is not clear-cut. For example, the development of therapeutics for depression is often performed in large and lengthy clinical trials which, if not carefully designed, may be characterised by high placebo-response rates, high drop-out rates, heterogeneous patient populations, and pharmacodynamic read-outs based on less robust subjective rating scales.

To overcome some of these caveats, PET is being increasingly used as a tool to determine drug dose–enzyme/receptor occupancy relationships in small and well-defined populations\textsuperscript{6,7}. The use of PET ligands that specifically bind to the target receptor or enzyme can provide vital information about: (i) the occupancy of the target site for a given dose of drug; (ii) the time-course of occupancy; and (iii) the relative plasma and tissue kinetics of the drug in question\textsuperscript{8}. Occupancy studies are performed with PET radioligands which are usually not identical to the drug candidate under study, e.g. the radioligand \([^{11}\text{C}]\text{WAY 100635}\) to determine pindolol occupancy at 5-HT\textsubscript{1a} receptors\textsuperscript{9}.

**Characterising the downstream biochemical consequences of drug action**

The occupancy of a drug at a receptor has downstream consequences for the neuronal signalling pathway that eventually manifest themselves as a pharmacodynamic response. In order to overcome some of the caveats associated with assessing drug effects using more subjective clinical end-points, downstream biochemical responses to drug action may be useful in determining the efficacy of drugs. Examples of downstream measures that can be used in this manner, are changes in glucose utilisation using \([^{18}\text{F}]\text{fluorodeoxyglucose (FDG)}\)\textsuperscript{10,11}, changes in cerebral blood flow using either \([^{15}\text{O}]\text{H}_2\text{O}\)\textsuperscript{11} or bold MRI\textsuperscript{12}, or probing a second messenger system directly (e.g. the cAMP cascade using \([^{11}\text{C}]\text{rolipram}\)\textsuperscript{13}). Many of the immediate post-receptor signalling pathways exhibit an inherent amplification of the original signal; for example, for every molecule interaction, several second messenger molecules are released. Thus probing the downstream consequences of receptor occupancy with a suitable second messenger probe may be a highly sensitive way of determining drug response even at low occupancy levels. This may be particularly useful where low occupancy of agonists are required to
generate a pharmacodynamic response. The consequences of drug action at remote neurons and receptors can often be observed, including the release of endogenous neurotransmitters (e.g. the effect of GABAergic compounds on dopamine receptors\textsuperscript{14}). The study of the downstream response of drug effects may also be a valuable tool in understanding drug tolerance, tachiphalaxis and post-receptor neuronal remodelling and plasticity.

Reverse pharmacology

Traditionally, drug targets are selected through prior knowledge of the involvement of a specific receptor or enzyme in a particular disease mechanism. However, with the characterisation of the human genome, a plethora of novel receptors and enzymes of unknown function are being discovered, and the biological role of these targets in man are unknown or poorly understood. Selective test molecules are now being developed in order to test the role of these new recognition sites as potential therapeutic targets for future drug development programmes, in effect reversing the traditional drug discovery process. This is termed ‘reverse pharmacology’. The use of non-invasive imaging methods to assess the expression and distribution of these targets in living man in health and disease at an early phase in the drug discovery process will be an invaluable tool in aiding the systematic development of this new generation of drugs.

Understanding and characterising human biochemistry in health and disease

One of the major hurdles to the diagnosis, treatment and management of neurological and psychiatric illness is developing a robust understanding of the related abnormal biochemistry which is present in these disorders. Many of the chapters in this book address specific diseases and aspects of dysfunction in related biochemical pathways. Although the understanding of some of these disease mechanisms is more developed than others, the complexity of the central nervous system means that our understanding of CNS disorders is relatively reductionist and primitive. There is an intimate relationship between understanding disease mechanisms, the diagnosis of the disease, development of drugs to treat it, and the subsequent management of the disease. The testing of novel and existing probes at novel and established molecular targets will allow the generation and testing of new hypotheses relating to CNS disorders and, in turn, increase our potential to diagnose, treat and manage these diseases. For example, with a rapidly growing
elderly population, there is a huge unmet need for the treatment of dementias (e.g. Alzheimer’s disease). The presence of amyloid plaques in post mortem brain tissue of Alzheimer’s patients has led to speculation that deposition of this protein in the human brain may be a key component in the pathophysiology of this disease. Recently, a number of PET ligands have been developed specifically to target amyloid plaques. These ligands have the potential to enhance our understanding of the natural progression of amyloid plaque deposition and its relevance to the disease in living man. The understanding of the biochemical mechanisms and time course of plaque deposition may allow us to study and diagnose Alzheimer’s disease many years in advance of the manifestation of clinical symptomology. Such an understanding will also allow the potential use of disease-modifying drugs in selected at-risk populations at a very early stage in the disease process and to monitor the efficacy of new drugs targeting the disease at a presymptomatic stage. The partnership between academic and industrial sectors in enhancing our knowledge of such disease processes is a prerequisite for developing our future capability to diagnose, treat, and manage human disease.

Conclusions

The human brain is a complex organ, consisting of millions of inter-communicating neurons. The understanding of biochemical abnormalities relating to diseases and disease processes is key to the future development of effective diagnosis and novel therapeutics. The study of biochemical abnormalities in living man is rapidly becoming an essential and integral component of the drug discovery and development process. To this end, the use of non-invasive imaging modalities (e.g. PET) is an invaluable tool for the development of drugs in the 21st century.

Key points for clinical practice

- PET imaging provides a non-invasive and quantitative assay of normal and abnormal neurochemistry in living man at an early stage of the drug discovery process to enhance the efficient and effective discovery of therapeutics.

- Tracer doses of labelled compounds enable the early evaluation of novel drugs in man: biodistribution studies; receptor/enzyme occupancy studies to optimise drug-dosing regimens; and characterising downstream responses of drug action.

- Understanding disease mechanisms in living man using non-invasive techniques is intimately connected with future developments in the diagnosis and management of disease, and of novel therapeutics.
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