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REVIEW ARTICLE

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# Positron emission tomography microdosing: a new concept with application in tracer and early clinical drug development

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**Abstract** The realisation that new chemical entities under development as drug candidates fail in three of four cases in clinical trials, together with increased costs and increased demands of reducing preclinical animal experiments, have promoted concepts for improvement of early screening procedures in humans. Positron emission tomography (PET) is a non-invasive imaging technology, which makes it possible to determine drug distribution and concentration in vivo in man with the drug labelled with a positron-emitting radionuclide that does not change the biochemical properties. Recently, developments in the field of rapid synthesis of organic compounds labelled with positron-emitting radionuclides have allowed a substantial number of new drug candidates to be labelled and potentially used as probes in PET studies. Together, these factors led to the logical conclusion that early PET studies, performed with very low drug doses—PET-microdosing—could be included in the drug development process as one means for selection or rejection of compounds based on performance in vivo in man. Another important option of PET, to evaluate drug interaction with a target, utilising a PET tracer specific for this target, necessitates a more rapid development of such PET methodology and validations in humans. Since only very low amounts of drugs are used in PET-microdosing studies, the safety requirements should be reduced relative to the safety requirements needed for therapeutic doses. In the following, a methodological scrutinising of the concept is presented. A complete pre-clinical package including limited toxicity assessment is proposed as a base for the regulatory framework of the PET-microdosing concept.

**Keywords** Positron emission tomography (PET) · Labelled drugs · Drug development

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## Introduction

Increased failure rate of new potential drug candidates and dramatically increased costs for the development of these drugs (costs borne by patients and society) [1] have initiated discussions on how to make drug development more effective. A number of scientifically valid proposals have been published in recent years to improve the drug development process and change the present empirical paradigm to a more mechanistic and predictive one [2, 3, 4, 5, 6].

The present cost for development of a new drug has been estimated at US $800 million. Attrition rates are still very high. More than 70% of candidate drugs entering clinical drug development fail [1]. Of all clinical research and development spent, 72% is spent on candidates that fail. Analysis of the process has identified two major causes: (1) poor predictability (of properties in man) of the preclinical models used for candidate selection and (2) insufficient learning before entering phase III.

At the same time, the revolutionary development in molecular biology has opened up a range of new potential targets for drug interactions, and combinatorial chemistry together with high-throughput screening methods has given an enormous range of chemical entities available for applications in various animal models up to humans.

In this dilemma, it has been discussed that the initiation of human studies should be simplified. Different options of low dose first in man studies as a candidate

screen have been proposed [7, 8, 9]. Such studies can by necessity only have a limited scope with the main perspective being the assessment of pharmacokinetics, but as such it might be one major indicator for selection or rejection of a drug candidate. In fact, inadequate pharmacokinetic properties constitute the major cause of failure of new drugs when probed in man. With the present strategies, human data will be available only after an extensive range of pre-clinical studies.

In the present paper, we describe a concept utilising modern imaging technology for studies in humans in the candidate selection process. By utilising a low dose (microdose) of a new chemical entity, labelled with a positron-emitting radionuclide with no effect on the chemical properties, positron emission tomography (PET; PET-microdosing) can be used to obtain crucial distribution and kinetics information in humans, some of which cannot be reached using any other method. The same concept would additionally be valid for the first human trials of a tentative new PET tracer, aiming at the in vivo characterisation of biochemical or physiological parameters.

## Positron emission tomography

PET is a non-invasive tomographic imaging method, which utilises pharmacological or biologically/biochemically active compounds labelled with short-lived positron-emitting radionuclides. These labelled agents are administered to a volunteer, a patient or a research animal, mostly as i.v. injections, and their fate in vivo is recorded by external detection and visualised as sets of tomographic images.

### Radionuclide production

The radionuclides are predominantly produced by charged particle nuclear reactions using a cyclotron, where a target container with a gas or a fluid is bombarded by protons or deuterons. A typical production includes the use of protons irradiating a target chamber containing purified nitrogen gas, leading to the generation of $^{11}C$ via the nuclear reaction $^{14}N(p,\alpha)\ ^{11}C$. The $^{11}C$ reacts with minute amounts of oxygen in the gas generating $^{11}CO_2$, which can be further processed by chemical reactions, for example to $^{11}CH_3I$, $H^{11}CN$ or $^{11}CO$, each of them being a potential building block for further syntheses. The major advantages of the positron-emitting radionuclides include:

- They can be included as normal constituents in a biologically active molecule without changing the molecule's biological property
- They can be measured externally using a PET camera, which has high resolution and sensitivity
- They have a short half-life, well fitted to the examination time relevant for human studies

The commonly used radionuclides in PET that are most appropriate for labelling of drugs are summarised in Table 1.

### Synthesis of labelled drugs

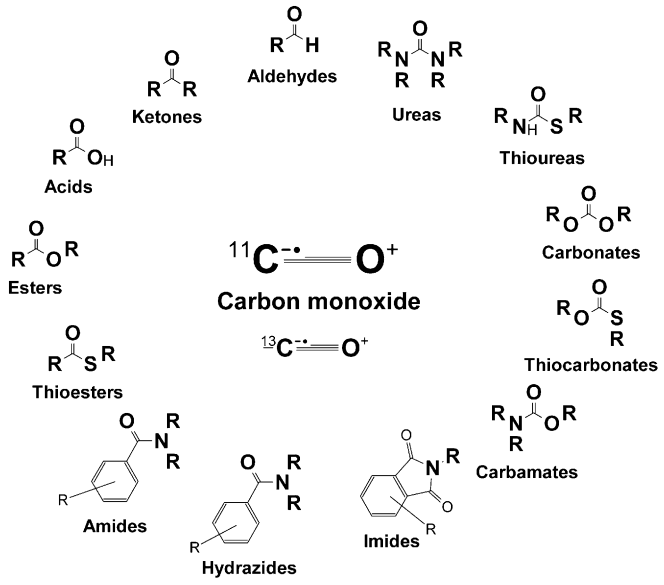
The main challenge to prepare drugs labelled with these short-lived radionuclides from the synthetic perspective is the short half-life of the radionuclides and the very low concentrations of the intermediate building blocks. Assuming that we have a drug with a molecular weight of 500 g/mol and will work with a specific radioactivity of 50 GBq/μmol and a total radioactivity in the order of 10 GBq means that the synthesis scale will be in the order of 0.2 μmol, which equals 100 μg.

The labelling synthetic methods allow introduction of these radionuclides into many organic molecules using for example the intermediate building blocks presented in Table 1. In the past, a typical strategy for labelling with $^{11}C$, would be to implement a methyl structure in the molecule using $^{11}CH_3I$ as the building block [10]. A quick survey of registered drugs indicates that 15–20% of these contain methyl groups in the structure. That means that the main part of the literature including $^{11}C$-labelled compounds until now is using a methylation strategy [10]. In the last few years, however, labelling strategies using $^{11}CO$ has opened up new avenues for labelling many functional groups [11, 12, 13, 14, 15, 16]. In scheme 1, examples of functional groups prepared via synthetic routes using $^{11}CO$ are shown. With these new possibilities, the concept of labelling most small molecules drugs has become a reality and is now more than a vision.

Utilising the appropriate chemical reactions and utilising the existing building blocks, many labelled compounds can be prepared. The labelling performance today is handled by an automated computer-controlled synthesis system, which allows flexibility and safety [17]. The production of these radiopharmaceuticals are today performed according to GMP procedures including verification of the identity and performing appropriate pharmacy to have the compound ready for administration in man (Scheme 1).

**Table 1** Characteristics of positron emission tomography radionuclides

| Radionuclide | Half-life | Production mode | Intermediate building blocks | Typical specific radioactivity (GBq/μmol) |
|---|---|---|---|---|
| $^{11}C$ | 20 min | $^{14}N(p,\alpha)\ ^{11}C$ | $^{11}CH_3I$, $H^{11}CN$, $^{11}CO$ | 10–200 |
| $^{18}F$ | 2 h | $^{18}O(p,n)\ ^{18}F$ | $^{18}F^-$ | 50–400 |
| $^{76}Br$ | 16 h | $^{76}Se(p,n)\ ^{76}Br$ | $^{76}Br^-$ | 50–200 |




**Scheme 1** Examples of functional groups generated from $^{11}CO$

## Scheme 1

### Examination

The labelled compound is in most cases administered to the subject via an i.v. injection or by an infusion. The small sample of injectate contains relatively high amounts of radioactivity (200–800 MBq) and, in order to avoid local radiation damage, it is essential that the injectate is not allowed to remain long at one locality, and i.v. injection is the preferred administration route. Oral administration or inhalation is possible but typically then the total dose of administered radioactivity must be reduced by a factor of ten, in turn leading to less precise measurements and noisier images. At the moment of injection, the camera is activated to start acquisition, meaning that the detectors are ready for monitoring of gamma rays. When a positron-emitting radionuclide is decaying, it emits a positron, a small electron-like particle with positive charge, that traverses tissue with multiple collisions like a small billiard ball, travelling on the average a distance in the order of 0.5 mm before being brought to rest. At this moment, it interacts with an electron in an annihilation event whereby these particles jointly are converted to two oppositely directed gamma rays of 511 keV energy. The gamma rays readily penetrate the tissue and a fraction of them emerge out of the body to be recorded by the PET camera.

The camera typically consists of a gantry with multiple rings of detectors, which measure gamma rays emerging from the subject. One gamma ray of the photons in the pair is hitting a detector on one side and the other one a detector on the other side, constituting one count. Typically counts in the order of 10 million are recorded, subdivided into different time periods after tracer administration. Computer processing of data generates sets of tomographic images, with typically 63 tomographic slices covering an axial extent of the body of 15 cm and with a spatial resolution of 5 mm. Each time period is defined as a separate image set, a frame, acquired for a predefined time length and at a predefined time after administration. The information in the images is quantitatively accurate and represents radioactivity concentration.

### Analysis of images

The images are displayed on a computer monitor for inspection, allowing the identification of areas of the body with different degrees of uptake of the tracer. Comparisons are made between images obtained at different times after administration.

The images are of definite interest but equally important is the possibility to obtain quantitative values on the drugs/tracers concentration–time profile in different organs or sub-regions of organs. For this, regions of interest (ROI) are manually outlined in the images to represent targeted regions for analysis. Within these ROIs, the radioactivity concentration is determined for each frame in the sequence, resulting in a time–activity data set, displayed as a time–activity curve. The radioactivity is rapidly decaying according to the half-life of the radionuclide and therefore a correction is applied to compensate for this. It is common in the PET field to recalculate radioactivity concentration into standardised uptake value (SUV), a dimensionless entity (actually ml/g but with an assumed tissue density of 1 g/ml interpreted as dimensionless; the transmission scan can determine the actual density allowing a correct determination), which compensates for differences in administered radioactivity and body weight.

– SUV = local radioactivity concentration/(administered radioactivity/body weight)

The time activity data express the tracer's kinetics in a region and can in turn be used for the assessment of the drug concentration if the study is performed in such way that tracer and drug are mixed and administered together. In this instance the relationship between cold compound and radioactivity is constant with time, if compensation is made for radioactive decay. Thus, also within each region, the relationship between radioactivity concentration and drug concentration is constant and equal to that between administered radioactivity and administered drug.

– Drug concentration = SUV×(administered drug/body weight)

An example: in a co-injection study, the subject of 75 kg was given 300 MBq labelled compound mixed with 100 mg cold compound. The PET study measured a SUV in the brain of 1.2. This means that the drug concentration in the brain was 1.2×(100/75) mg/kg = 1.6 mg/kg = 1.6 μg/g.

The time–activity data of regions, combined with plasma time–activity data can further be used in pharmacokinetic modelling in order to define exchange parameters and assess steady-state distribution volumes of the drug in organs.

## PET-microdosing

The labelling of compounds with short-lived radionuclides usually results in super-high specific radioactivity, i.e. the amount of radioactivity per unit of cold compound is very high, in the order of 10–400 GBq/μmol. The amount of radioactivity needed for a high quality investigation is typically in the order of 300–800 MBq. Radiation safety considerations typically limit the total amount of radioactivity given to a healthy volunteer to about 2000 MBq, an amount which can be subdivided into more than one investigation. The counting limitations of the camera typically limit the amount of administered radioactivity to about the same amount.

A PET study performed with the administration of 600 MBq would be associated with typically 6–20 nmol drug, corresponding to 3–10 μg (assuming a MW of 500).

With these minute amounts of drug administered to a human, very important information can be obtained regarding pharmacokinetics, organ distribution, access to target organ and potential unexpected accumulation in side organs.

The administration of minute amounts of a new chemical entity in humans in single acute experiments would require a risk assessment and therefore a pre-clinical toxicity evaluation must be at hand. However, it is our view that this toxicity assessment should be limited relative to what is required for a drug given in large amounts and to larger groups of individuals. In the following, we present a basis for toxicity assessment prior to a PET-microdosing study (not yet accepted by regulatory agencies). While detailed requirements should be considered on a case-by-case basis, depending on the properties of the candidate as well as the intended target, a common preclinical package is suggested below. It is essential that specific technical and scientific aspects for the interpretation of the results and their applicability to human drug distribution is covered and, for this reason, the package includes different aspects of validation of the PET procedure for each specific new chemical entity.

## Preclinical toxicity requirements

The EMEA committee for proprietary medicinal products (CPMP) has issued a: "Position paper on non-clinical safety studies to support clinical trials with a single microdose" (http://www.emea.eu.int/pdfs/human/swp/259902en.pdf), which specifies the EMEA requirements for safety studies related to PET-microdosing. In the following, we summarise these requirements.

The ICH M3 recommendation is for safety pharmacology, single-dose toxicity studies and repeated-dose toxicity studies. This set of studies may be replaced by an extended single-dose toxicity study in one mammalian species if the choice of species could be justified based on comparative in vitro metabolism data and by comparative data on in vitro primary pharmacodynamics/biological activity.

The extended single-dose toxicity study should include a control group and a sufficient number of treatment groups to allow estimation of the dose inducing a minimal toxic effect. For compounds with low toxicity, a limit dose approach could be used. Allometric scaling from animal to man using a safety factor of 1000 should be used to set the limit dose. Both genders should be considered. The study period should be 14 days and include an interim sacrifice on day 2. The study should be designed to obtain information on haematology and clinical chemistry at a minimum of two time points (day 2 and day 14) and histopathology.

In addition, all available background information on the test substance and/or close pharmaceuticals as well as on the therapeutic class with respect to vital organ function and other safety parameters should be supplied. Examples are receptor screening profiles, activity at HERG, effect on action potentials etc. In vitro genotoxicity studies should be performed as recommended in relevant ICH guidance.

### Comments to the CPMP guidance on genotoxicity

The tests are performed in accordance with the standard regulatory requirements. However, we suggest the doses used to be adjusted to match the conditions when microgram doses will be administered. Additionally, it is our experience that it is seldom possible to formulate modern drugs up to the concentrations defined in the present regulations. Finally, the amount of drug needed for genotoxicity assessment in the present form is in the order of several grams, and these amounts are usually only available later in the drug development process. Hence, we suggest that only three doses of the test substance be applied, the maximum being 5 μg/plate, which equals a test concentration of 2.5 μg/ml.

A PET tracer is administered to humans at a typical amount of about 5 μg and is given as a single dose or in as few doses to the same individual (radiation dose is the limiting factor). The previous requirement of testing up to 5 mg/plate would use an effective concentration in the assay amounting to 25,000,000 times higher than the effective concentration in a human PET study. This safety margin is far beyond reasonable, also in view of the fact that the major health concern in a PET study would be the radiation dose associated with the tracer. A PET study is typically associated with radiation exposure giving an average radiation dose of about 5 mSv. A safety margin of 25,000,000 on drug concentration would, if given as a PET tracer, be associated with a radiation dose of 125.000 Sv,

which is about 10,000 times higher than a lethal whole body dose!

### Radiotoxicity of tracers

A group of test animals are given the tracer at typical expected human or higher tracer concentrations. Animals are sacrificed at different time points and different organs and blood removed and analysed with respect to radioactivity concentration. These data are used as general information with respect to the tracer's behaviour in the body, including organ distribution and time course of accumulation and elimination.

These data are additionally used to give an estimate of radiation doses that can be expected in different organs in a human study. Especially important is to exclude the possibility that selective accumulation in a radiosensitive organ could lead to radiation-induced damages. The average radiation exposure in a PET study is typically equivalent to the natural background radiation achieved during a few years, about 2–4 mSv, which in turn is of the same order as a body CT investigation. The measurement of blood volume adds about 1–2 mSv.

## Validation requirements

The purpose of a PET study is to supply information on the drug's distribution pattern and kinetics with the potential to use this information for the acceptance or rejection of a candidate drug. This would imply that we must make it likely that the PET study can be performed technically adequate, that the information obtained is correctly reflecting the conditions of the drug and is relevant not only to drug at microdosing concentrations but also at assumed therapeutic concentrations. For a potential tracer, the validation would include to verify that the tracer would have appropriate properties to define the target system with sufficient specificity and precision. To ensure these aspects, a few preclinical evaluations are recommended.

### Primary pharmacology

The primary pharmacology should be available including suggested target system, affinity for this target and specificity. Affinity for other targets can be important to know in order to allow an estimate if other selective binding sites could influence the tracer distribution at tracer concentrations.

### Metabolism and appearance of labelled metabolites

Since PET can only measure radioactivity and not discern the chemical form of it, it is not possible in a PET study to be certain that the radioactivity signal is related to the original compound or including metabolites thereof. In order to make it likely that the proper conclusion is drawn, it is recommended that pre-clinical studies be performed in which the fraction of radioactivity constituted by intact tracer is determined in plasma and in the target organ. Additionally, the plasma profile of radioactive metabolites should be assessed in the human PET-microdosing study. Such studies are best planned if knowledge of the metabolic pathways is available.

A typical example of such study is that rats are injected with relatively high amounts of radioactivity. At certain time points they are sacrificed and plasma and target organ is removed, prepared and analysed with high-performance liquid chromatography (HPLC) with radiodetection or sampling of radioactive peaks. In addition, the development of highly sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS) technology could enable the measurement of the tracer in blood/plasma as a validation of the PET signal [7, 8, 9, 18].

### Dose linearity

In a PET study, the average body concentration after injection is in the order of 0.1–0.3 nM with plasma concentration decreasing with time. These conditions are very different from those existing at a therapeutic dose with more than 1000 times higher concentrations. Many enzymes systems and receptor-mediated transport systems can be saturated at higher concentrations and drug distribution at very low concentrations can be governed by high-affinity binding sites. This implies that it is quite possible that the distribution, metabolism and elimination pattern can be different at tracer doses and therapeutic doses. However, the PET method allows the recording of tissue kinetics and plasma kinetics at the same time and, therefore, allows a modelling of this relationship. It is our experience that the exchange of drug between tissue and plasma is usually little affected by dose and more conserved in-between species. So, with dose-linearity, we mean that the tissue-to-plasma relationship is unaffected by dose. In order to allow a correct extrapolation from a PET-microdosing study, it is necessary to make an assessment of dose linearity. This would be performed as a rising dose PET study in a monkey or other research animal, going from a tracer alone administration to a therapeutic dose mixed with the radiolabelled counterpart. Plasma kinetics and organ kinetics is recorded and additionally the fraction of non-metabolised tracer in plasma is measured.

With dose linearity, the time–activity data of each of the doses would be the same, and, if so, it would be assumed that dose linearity is at hand also in a human PET-microdosing study. Even lack of dose-linearity could be handled, if it can be described well, or if, e.g., the tissue–plasma exchange parameters are dose independent.

### Validation of a tracer for a target system

To ensure that a tracer correctly measures a target system and interactions to it, a relatively large set of studies need to be performed, including in vitro assays and in vivo animal studies. The binding properties should be explored with respect to association, dissociation, specificity, etc. Methods to acquire a quantitative value of the desired parameter should be defined and tested in vivo in animal PET studies and confirmed to:

- Correlate anatomically to in vitro assays
- Give an expected dose-dependent pharmacological effect by structurally different agents
- Not be affected by pharmacological agents that do not interfere with the target
- Not be affected by induced changes in blood flow

### Technical aspects

Prior to the initiation of a human study, it is also desirable to probe some technical aspects and assumptions leading to modifications in the investigation set-up. A PET study in monkey, which gives technically adequate measurements is a safe background to the human study. Such a study can aid in defining the optimal time points for scanning and blood sampling. Sometimes a very high uptake in an organ close to the target organ can make it difficult or impossible to study this target organ, e.g. a very high uptake in liver can exclude the possibility to measure tracer kinetics in the pancreas. Similarly, a very high uptake at the administration site can severely limit the imaging in adjacent structures, as, e.g. with a nasal administration.

## PET-microdosing study

A PET microdosing protocol would by necessity have to be accepted by the national Regulatory Agency, by the Institutional Review Board and the Local Radiation Safety Board. A protocol would typically include in the order of six healthy volunteers. The scanning protocol would include scanning over one sector of the body of special interest or with successive movements between a few sectors of the body. A sector is in the order of 15 cm in axial coverage and if kinetic sampling in one sector can be compromised, movement between sectors and thereby a lower temporal sampling can be performed. The order of measurements between different anatomical regions can be changed between individuals.

The total examination time is limited by the half-life of the radionuclide, being in the order of 90 min for a $^{11}$C-labelled drug. During the study, multiple blood samples are taken for measurement of whole blood and plasma radioactivity and some of them used for HPLC analysis of fraction of intact tracer. As a complement, samples can be sent for LC/MS/MS or accelerator mass spectrometry (AMS) analysis of drug concentration [7, 8, 9]. We wish to emphasise that high sensitivity methods for plasma pharmacokinetic (PK) analysis and low administration doses are not competitive but complementary to PET-microdosing. The former can give very important late PK which is not available for PET, whereas the latter can give organ kinetics not accessible by the former method.

### Correction for vascular tracer

With some drugs with poor entry to the target organ, a significant part of the observed tracer concentration in this organ can come from tracer in the vasculature. To obtain the desired entity, drug concentration in organ parenchyma, it may be necessary to subtract the contribution from drug in the vasculature. This is readily done if the PET sequence includes a separate scan for the measurement of local blood volume. This is performed in 10 min with the inhalation of $^{15}$O-CO for labelling of red blood cells, measurement of regional radioactivity concentration with the camera and relating these measurements to blood radioactivity concentration. The result is local blood volume expressed as a percentage of volume constituted by blood. In the drug PET-microdosing study, radioactivity concentration in blood is determined at different time points, and by multiplication of these values with regional blood volume, the vascular contribution is determined and subtracted.

## Examples of PET-microdosing examinations

The new paradigm with PET-microdosing as a first in man study is still under discussion, although the Swedish Medical Product Agency and CPMP have agreed on the general concept discussed above. However, some further refinements have been made by us as indicated above. We have already performed a range of PET studies with administration of microdosing levels of drugs under development. Several of these compounds have been complemented by PET studies in the same individual with administration of tracer plus drug. Hence, we have available a range of studies in which the validity of the assumption of dose linearity from tracer doses to therapeutic doses can be probed (Fig. 1, Fig. 2, Fig. 3).

In the first example, three different drugs targeted for the brain were explored, and one proven to have a very high and rapid brain entry, one showed limited but still adequate brain penetration and one had no measurable brain entry. In the latter case, the drug did not even enter extracranial tissues lacking blood–brain barrier, which is explained by an extremely high and in the time lines of a PET study not reversible plasma protein binding.

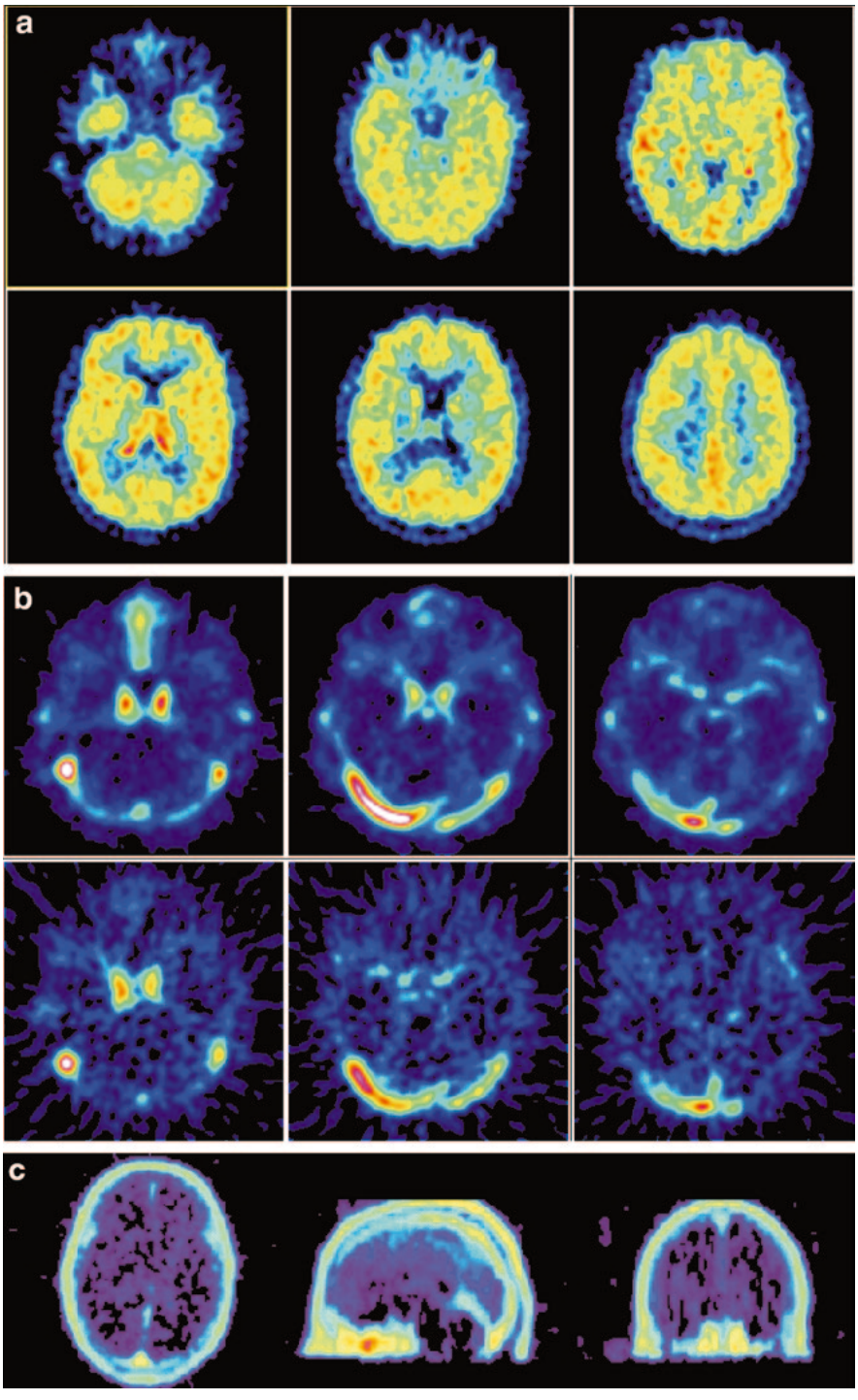



**Fig. 1** **A** Brain distribution of a labelled NK1-receptor antagonist drug, with high brain uptake. The time course is slightly different at tracer doses and therapeutic doses while at tracer doses a significant part of the tracer is bound to NK1-receptors, but the tracer alone study, with focus on brain regions without selective receptor binding predicted well the brain concentration at therapeutic doses. **B** Brain uptake of a drug with indication acute brain damage, showed lack of entry to the brain parenchyma. This was observed both at tracer doses and at therapeutic doses, indicating the value of the tracer alone study. The lower images show blood-volume images of the same subject, obtained with PET and $^{15}$O-CO-labelled red blood cells. The lack of tissue entry also in extracranial soft tissue suggests a tight binding to blood components which does not allow exchange with tissues. **C** Brain uptake of another drug aimed at acute brain damage, show some uptake in brain and good exchange with extracranial tissues, indicating that a restricted uptake in brain is motivated by the blood-brain-barrier. It can be expected that perturbed areas with compromised blood-brain barrier would have high drug concentrations

Another example is a drug that has a very high uptake in the adrenal gland due to its selectivity for 11$\beta$-hydroxylase [19]. Studies in monkey proved that this uptake also was at hand at therapeutic doses, implying that substantial concentrations can be expected in the adrenals in a treatment study. Clinical and experimental studies have shown that this drug can promote even life-threatening adverse effects by blockade of cortisol synthesis.

## Discussion

The basic concept behind PET-microdosing is not new; we have already 1984 published PET studies with the rational to use PET to explore the distribution/elimination of new drugs [20, 21, 22, 23, 24, 25, 26]. However, the concept has become a general potential with the advent of new synthesis methods, allowing a majority of

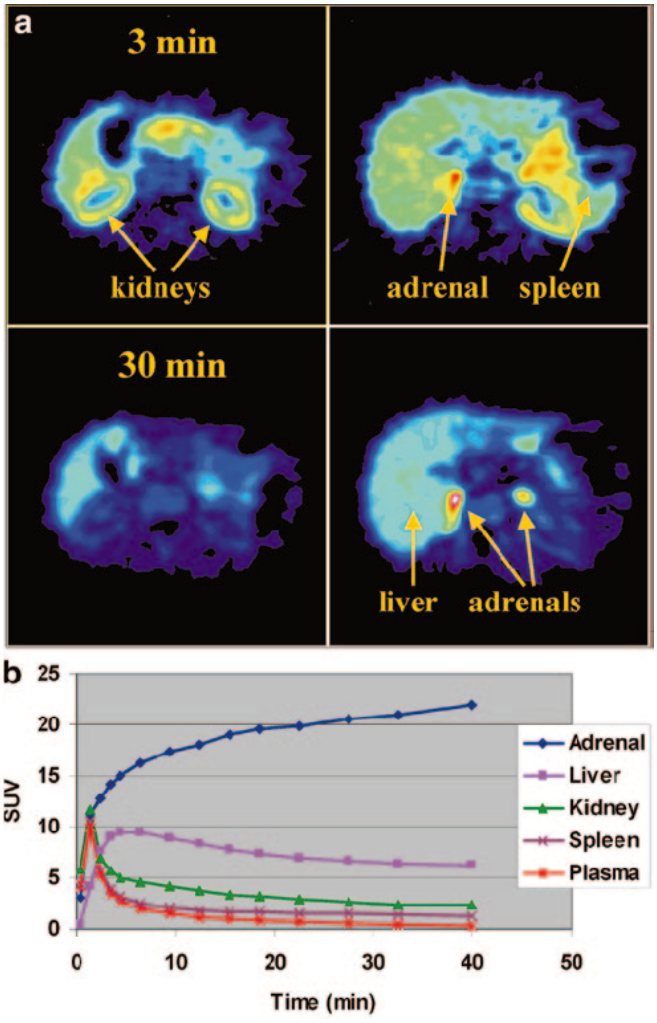



**Fig. 2** Axial slices obtained with $^{11}$C-labelled metomidate, a close analogue of the anaesthetic etomidate, which was found to have very high affinity for 11$\beta$-hydroxylase in the adrenal cortex. The concentration in the adrenals increases linearly with time to give tissue-to-plasma ratios of more than 50

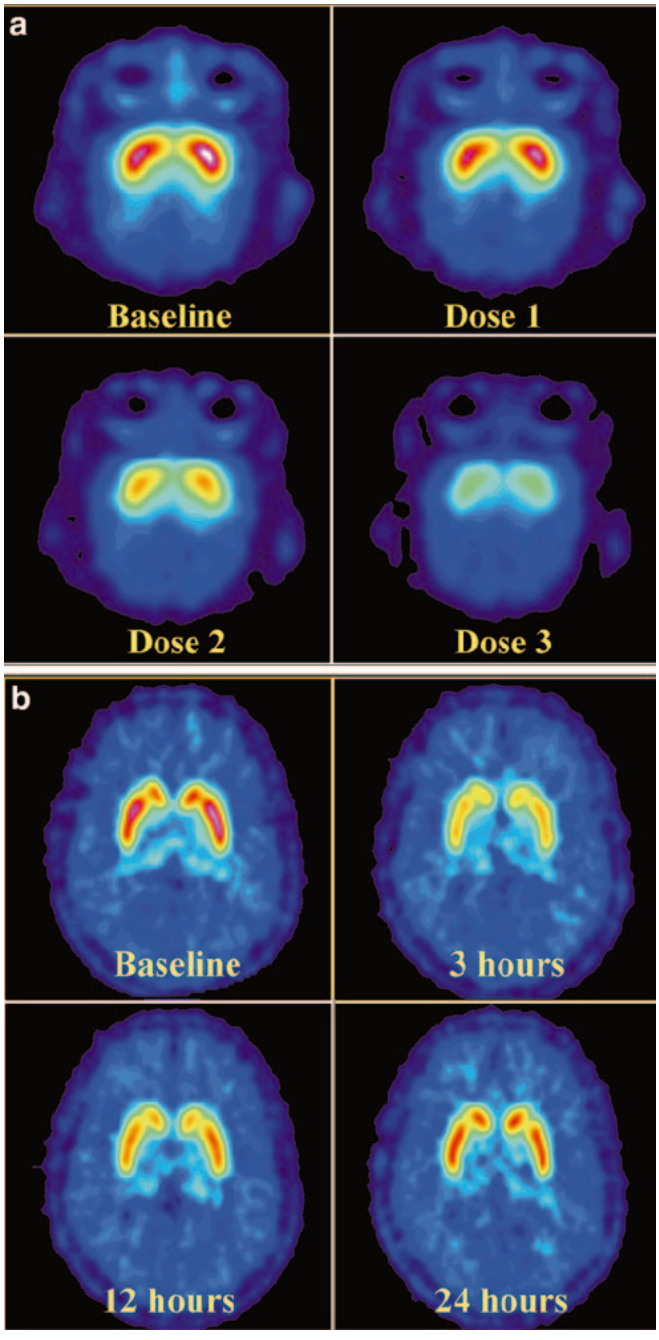



**Fig. 3** The dose-occupancy relation in Rhesus monkey **a** for a drug with the dopamine transporter as a target is evaluated with the tracer $^{11}$C-beta-CIT-FE and used for the prediction of dosing in humans **b** In this case the drug undergoes full tox evaluation since it is used in humans at therapeutic doses, whereas the tracer undergoes the limited tox of the PET-microdosing package

new drug candidates to be labelled [27, 28, 29, 30]. Of special importance in this respect has been the introduction of $^{11}$C-carbonmonoxide as a synthon [11, 12, 13, 14, 15, 16]. This has opened up a significant increase in the synthesis potential. A second feature which has been of importance for the promotion of PET-microdosing is the increased use of PET in drug development where the potential to follow drug distribution and drug interaction with target systems has really gained acceptance in drug industry.

The possibility to assess drug pharmacokinetics in early trials in humans is of great importance in drug development and can supply vital information, which can disqualify a drug candidate at an early stage. By this, a range of studies in animals can be avoided and direct the focus on follow-up candidates. Although molecular biology has had an explosive development, methods in this field can usually not predict well the drug behaviour in humans, especially with respect to quantitative aspects. Hence, human trials remain the cornerstone for selection or rejection. Except for supply of selection information based on pharmacokinetics, a PET-microdosing study can be of additional value by giving information about selective accumulation in organs, which are not target organs. Very high accumulation in one organ can put attention on the possibility of side effects, which can be further explored. The distribution pattern and kinetic

profile in humans can also give guidance with respect to selection of animal species to be used in safety assessments. The major application of PET-microdosing for candidate selection, which has been discussed till now, has been for neuroactive drugs with the question of passage over the blood–brain barrier. However, more important has been the drive to have available PET tracers for assessment of interactions with target systems. This task is more on the critical path of drug development and puts a high strain on tracer development and validation and needs a human confirmation before it can be used. This confirmation including assessment of test–retest variability is optimally performed as a PET-microdosing study since the tracer candidate is usually not a drug candidate.

A PET-microdosing study can per se not give information regarding the dose occupancy relationship since it is only performed at very low concentrations. Selective binding of the tracer can, however, depending on the properties of the tracer, sometimes be seen. A verification of specific binding would in the general case depend on a comparison of data from a tracer-alone study and a study in which the target has been blocked pharmacologically. In some cases, selective binding can be assessed by comparison of tracer kinetics in a sub-region expressing a receptor with a sub-region with similar perfusion properties but lacking the receptor. Finally, in some cases, pharmacokinetic modelling of the tracer kinetics in a target region with consideration to plasma tracer kinetics can assess selective receptor binding. In all of these cases, it would be necessary to back up the studies with a range of validation studies in animals.

The concept of PET-microdosing should be seen as a whole package including assessment of safety aspects, preclinical validations and performance and analysis of human trials. It is important to consider that the drug selected for a PET-microdosing study at this stage of use is only intended to be given at very low doses, with a single or only a few administrations and to a small and well-controlled population. These factors suggest that the safety rules applied to a drug for general and long-term use at high doses are not relevant. One important aim of non-clinical studies is to reduce the chance of administering to humans a compound that has unacceptable risks. However, this must be critically balanced against the increasing demand within society to reduce or preferably eliminate animal studies. In this context, PET-microdosing contributes to reducing animal testing.

From safety aspects, there is no major difference between the probing of a new drug using PET-microdosing paradigm and the validation of a new PET tracer to be used for assessment of biochemistry/physiology. We therefore suggest that the PET-microdosing package described above should also be relevant in the process of generating a new PET tracer.

PET studies can, if well planned in the drug development process, be very cost effective, although the price of PET may seem high. A very rough estimate of the costs of this PET-microdosing package suggest an overall cost of about US $300,000, subdivided into chemistry development $40,000, safety evaluation $50,000, preclinical validation $50,000, PET studies $110,000 and CRO costs $50,000.

The suggested paradigm shift in drug development from an empirical to a more mechanistic and predictive scientific mode [31, 32] is supported by PET-microdosing. Hence the PET-microdosing study can be used together with the monkey distribution data to generate models for the relationship between plasma and target tissue concentration. These models can then be further worked out to include aspects of receptor occupancy, which is backed up with monkey dose escalation studies with observation of occupancy. Finally, human occupancy studies are used for the final refinement of the PK/pharmacodynamic (PD) modelling. The combination of a PK/PD driven process with mechanistic information will hopefully drive the development process towards an increased learning mode thereby improving cost-effectiveness.

A bearing principle for our suggestions is that at the stage of development of a new chemical entity, at which PET-microdosing is of interest, the compound is still not available in high amounts. The conventional safety tests would require the synthesis of several grams of the compounds, which would in turn take considerable resources and time. With the concept we proposed, the bulk of compound needed would be in the order of a few milligrams.

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