A method of studying pharmacokinetics in man at picomolar drug concentrations

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- 1 We describe a new method that enables the tissue kinetics of picomolar concentrations of drugs to be measured in man. The method is based on the administration of a drug, labelled with a short-lived positron-emitting radioisotope, such as carbon-11 ($t_{1/2} = 20.4$ min, $\beta^+ = 99.8\%$) or fluorine-18 ($t_{1/2} = 109.8$ min, $\beta^+ = 96.9\%$), which is then detected *in vivo* by an array of 10 large uncollimated sodium iodide scintillation detectors, arranged as five opposing pairs, with each pair collecting data over one major organ or region of the body.
- 2 To illustrate the scope of the new method we report the results of administering [*O*-methyl- ^{11}C]-diprenorphine, an established radioligand for central opiate (μ , κ , and δ) receptors and L-6- ^{18}F -fluoro-DOPA, a marker for dopaminergic neurons.
- 3 Only 2–10 μCi (74–370 kBq) of radioactivity are used and, as a consequence of the high specific activities with which carbon-11 and fluorine-18 labelled compounds can be prepared, the method requires less than a nanomole of drug to be administered. In many cases, this amount of drug might be considered low enough to avoid any adverse biological effect. Furthermore repeat studies are possible in man without delivering unacceptable radiation burdens.
- 4 The high sensitivity realised for both radioactivity and mass suggests a means for determining the human biodistribution of a new drug at a very early stage in its development. This has potential benefit to drug discovery programmes and to ensuing drug therapies.

Keywords pharmacokinetics whole body counting positron-emitting isotopes

Introduction

In the course of our developmental work in Positron Emission Tomography (PET) we have needed to explore alternative means of recording the distribution of drugs, labelled with positron-emitters, in man. It was reasoned that, if radioactivity in the liver and other organs could be monitored after injection of the labelled radiopharmaceutical, factors influencing metabolism of the radiopharmaceutical, such as diet, could be examined. Such information might identify conditions that minimise metabolism and the contaminating effects of circulating metabolites, and so lead to maximal delivery of the radiopharmaceutical to the organ of interest (e.g. brain, heart or lung) and thereby to optimal statistical quality in the PET scan. A procedure delivering a low radiation burden was required if serial studies of regional pharmacokinetics were to be performed safely in human volunteers. This need provided the initial stimulus for the development which is now described.

A positron-emitting radioisotope is externally detectable from the opposing pair of 511keV γ -rays that are generated from the annihilation of the emitted positron with a nearby electron. Some positron-emitting radioisotopes, by virtue of their short half-lives and means of production, are obtainable in high specific radioactivity for biomedical application. Important examples are carbon-11 ($t_{1/2} = 20.4$ min, $\beta^+ = 99.8\%$) and fluorine-18 ($t_{1/2} = 109.8$ min, $\beta^+ = 96.9\%$), which are widely produced in high activities by cyclotron-promoted nuclear reactions. In recent years, fast techniques have been developed for the synthesis of carbon-11 and fluorine-18 radiochemicals at high specific radioactivity (e.g. of the order of $\mu\text{Ci pmol}^{-1}$). These techniques now permit a high proportion of drugs to be labelled *isotopically*, so leaving biological properties essentially unchanged (see Fowler & Wolf, 1986; Pike, 1988 for reviews). To obtain accurate spatial delineations in human tissues using PET,

2–10 mCi (74–370 MBq) of labelled drug usually need to be administered. Even with the latest generation of PET scanner, only one region of the body, extending over an axial length up to 10 cm, can be monitored at any one time.

Whole body counters have been used to monitor the whole body retention (Sargent, 1962; Suguru *et al.*, 1963) or distribution (Trott & Cottrall, 1965) of low activities of administered γ -emitting radionuclides, including calcium-47 (Sargent, 1962), iron-59 (Sargent, 1962), strontium-85 (Suguru *et al.*, 1963), bromine-82 (Howe & Ekins, 1963) and iodine-131 (Trott & Cottrall, 1965), generally in simple chemical forms or as labels for endogenous compounds. Also, in one study (Sargent, 1962), a whole body γ -ray spectrometer has been used to detect low activities of positron-emitting carbon-11, induced in the tissues of patients irradiated with a 910 MeV α -particle beam. Here we describe the use of a highly sensitive detector system, normally used for whole body activation analysis (Spinks, 1979) to address the problem of assessing the whole body distribution of pharmaceuticals that are labelled with positron-emitting radioisotopes, such as carbon-11 and fluorine-18.

Methods

Radio pharmaceuticals

[O-methyl- ^{11}C]-Diprenorphine Carbon-11 was produced as [^{11}C]carbon dioxide by the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ reaction on nitrogen gas, using a Scanditronix MC 40 (mark II) cyclotron, and used to label diprenorphine in the O-methyl position (Figure 1) (Luthra *et al.*, 1990).

Figure 1 The structures of [O-methyl- ^{11}C]-diprenorphine and L-6-[^{18}F]-fluoro-DOPA.

Radiochemically and chemically pure [^{11}C]-diprenorphine (circa 80 mCi; 3 GBq) was obtained as a solution in sterile apyrogenic saline for injection with a specific radioactivity of 300 $\mu\text{Ci nmol}^{-1}$ (11 MBq nmol^{-1}) at the end of radiosynthesis (45 min from the end of radioisotope production).

Normal healthy volunteers were injected intravenously with circa 10 μCi (370 kBq) of [^{11}C]-diprenorphine at a specific activity of 150 $\mu\text{Ci nmol}^{-1}$ (5.5 MBq nmol^{-1}) in 5 ml of saline. This radioactive dose therefore corresponds to the coinjection of about 1 pmol of stable diprenorphine per kg body weight. All subjects were fully informed as to the nature and purpose of the study and gave their consent. Administration of Radioactive Substances Advisory Committee and local Ethics Committee permissions were obtained for these studies.

L-6-[^{18}F]-Fluoro-DOPA Radiochemically and chemically pure L-6-[^{18}F]-fluoro-DOPA (5 mCi; 185 MBq) (Figure 1) was prepared as a solution in sterile apyrogenic potassium dihydrogen phosphate solution (0.07 M) with a specific activity of 162 $\mu\text{Ci } \mu\text{mol}^{-1}$ (6 MBq μmol^{-1}) at the end of radiosynthesis (2 h from the end of radioisotope production) (Pike *et al.*, 1990). Subjects were injected intravenously with ca 2 μCi (74 kBq) of tracer at a specific activity of 150 $\mu\text{Ci } \mu\text{mol}^{-1}$ (5.5 MBq μmol^{-1}).

Radioactivity detection

The whole body counter comprises 10 large (15 cm diameter by 10 cm deep) sodium iodide scintillation counters. The detectors were arranged as opposing pairs above and below the subject. Data were collected from five regions chosen to include brain, heart, liver, bladder and muscle (in legs). The arrangement is illustrated diagrammatically in Figure 2. All detectors and the subject of study were housed in a shielded room made from 15 cm thick low activity steel. Background levels of radioactivity at each detector were determined with the

Figure 2 The arrangement of the five pairs of opposed sodium iodide detectors within the whole body counter for the detection of radioactivity in brain, heart, liver, bladder and legs, after administration of a carbon-11 or fluorine-18 labelled compound.

subject in place and prior to the administration of radioactivity. The field of view of a single detector is 45 cm full width at half maximum, as measured with a radioactive source placed below 20 cm of water. Typically, the distance between opposed detectors was 40 cm in this study. The large field of view and high sensitivity, combined with the low background, enabled regional distribution and uptake kinetics to be followed with ease for up to 2 h, following the injection of only 10 μCi (370 kBq) of carbon-11 labelled drug, or 2 μCi (74 kBq) of fluorine-18 labelled drug. The detector configuration recorded 4.7% of the total disintegration rate in the human body. Spectra (200 keV–3 MeV) were collected from each pair of opposed detectors over small time intervals of 1 min and 5 min up to 2 h from injection. Each spectrum was integrated over the 511 keV peak. Background was subtracted from the data and the geometric mean for the paired detectors was calculated.

Results

Figure 3 shows typical regional kinetics for the uptake of radioactivity, as recorded by the five pairs of opposed detectors, following the i.v. administration of [*O*-methyl- ^{11}C]-diprenorphine, an established radioligand for central opiate (μ , κ , and δ) receptors (Frost *et al.*, 1990). Of particular interest is the rapid uptake and retention of radioactivity seen by the pair of detectors recording data from the head. This data has the same pattern as the brain uptake of radioactivity recorded fully quantitatively with a PET scanner after an i.v. injection into man (Jones *et al.*, 1987) of a much higher activity (10 mCi; 370 MBq) of [^{11}C]-diprenorphine, labelled (Luthra *et al.*, 1985) in a different position, the *N*- α -methylene carbon of the cyclopropylmethyl group, at a specific activity of *ca* 190 $\mu\text{Ci nmol}^{-1}$ (7 MBq nmol $^{-1}$). Figure 4 shows the sets of data collected by the pair of detectors placed on either side of the head in three consecutive studies in the

Figure 3 Time course of radioactivity detected in the five paired positions illustrated in Figure 2 following intravenous injection of 10 μCi (370 kBq) of [*O*-methyl- ^{11}C]-diprenorphine at a specific activity of 150 $\mu\text{Ci nmol}^{-1}$ (5.5 MBq nmol $^{-1}$). Data are decay-corrected to the time of administration.

Figure 4 Time courses of radioactivity detected by the pair of detectors positioned over the head (see Figure 2) following intravenous injection of [*O*-methyl- ^{11}C]-diprenorphine at a specific activity of 150 $\mu\text{Ci nmol}^{-1}$ (5.5 MBq nmol $^{-1}$) from three consecutive studies on the same subject. In one study ($\square$) naloxone was administered to the subject at 40 min as described in **Results**. Data are decay-corrected to the time of administration and normalised to an injection of 10 μCi (370 kBq) in each case.

same subject. In one study the opiate receptor antagonist, naloxone, was administered intravenously as a bolus (1 mg kg $^{-1}$) then as a constant infusion (1 mg kg $^{-1}$ over 20 min) at 40 min after injection of the [^{11}C]-diprenorphine. Upon administration of the naloxone there was a progressive fall in the radioactivity detected by the pair of detectors over the head (Figure 4), while other pairs of detectors recorded no such abrupt change (data not shown). This result is consistent with previous PET data (Jones *et al.*, 1987), showing specific displacement of [^{11}C]-diprenorphine from brain opiate receptors by naloxone, and demonstrates the specificity of the recorded signals for this organ. By making use of the quantitative PET data obtained with this radioligand in human brain, the cerebral concentration in this study is estimated as being maximally about 4 pM, reducing to about 1 pM at 60 min. Even at the end of this study count rates were between 5 (for legs) and 33 (for liver) times background. The data collected up to 40 min in each study illustrate the achieved reproducibility.

L-DOPA (L-3,4-dihydroxyphenylalanine) crosses the blood-brain barrier and as a precursor to dopamine is used in the therapy of pathological states, such as Parkinson's disease, where the striata are deficient in dopamine. The fluoro analogue, L-6-[^{18}F]-fluoro-DOPA, is used in conjunction with PET to delineate loss of striatal neurons in patients with movement disorders (Nahmias *et al.*, 1985) and recently to monitor functional recovery after brain transplant of foetal tissue (Lindvall *et al.*, 1990). The regional tissue kinetics, as recorded with the array of scintillation detectors, revealed excretion of radioactivity into the bladder and relatively low accumulation in the head (Figure 5a). Figure 5b shows data obtained from the same subject scanned again after receiving an inhibitor of peripheral aromatic amino acid decarboxylase, namely carbi-DOPA (100 mg at 1 h before intravenous injection with L-6-[^{18}F]-fluoro-DOPA

Figure 5 Time courses of radioactivity detected in the five paired positions illustrated in Figure 2 following intravenous injection of L-6-[^{18}F]-fluoro-DOPA at a specific activity of $150 \mu\text{Ci mmol}^{-1}$ ($5.5 \text{ MBq mmol}^{-1}$) in two consecutive studies of the same subject. a) No pretreatment. b) Pretreatment with carbi-DOPA as described in Results. These data are decay-corrected to the time of administration and normalised to an injection of $2 \mu\text{Ci}$ (74 kBq) per subject.

and 50 mg at the beginning of the scan). This paired study illustrates reduced renal excretion of radioactivity and enhanced accumulation of radioactivity in the head as a result of the increased availability (Leenders *et al.*, 1989) of unmetabolised L-6-[^{18}F]-fluoro-DOPA in the periphery (Melega *et al.*, 1990). At the end of this study count rates were from between 31 (for legs) and 92 (for liver) times background.

Discussion

The effective dose equivalents associated with the administration of $10 \mu\text{Ci}$ of carbon-11 and $2 \mu\text{Ci}$ of fluorine-18 are of the order of $2 \mu\text{Sv}$ (see 'Radiation Dose to Patients from Radiopharmaceuticals'. *Annals of the ICRP*, 18 (1987)). The method described here for investigating pharmacokinetics therefore uses quantities of radioactivity that can be regarded as safe for repeat administration to normal healthy volunteers. Furthermore, these very small amounts of radioactivity, when coupled to the achievable high specific activity, mean that only very small amounts ($< 70 \text{ pmol}$) of stable drug need be co-administered. Thus it is possible to follow the regional organ kinetics of an administered drug at picomolar tissue concentrations, which in many cases would not be expected to provide a pharmacological effect. Indeed, this method would appear to be the most sensitive for drug detection *in vivo*. By exploiting known pharmacological mediators, such as naloxone and carbi-DOPA, it has further been demonstrated that this method can identify global and organ specific changes in the tissue retentions of the labelled compounds (Figures 4 and 5). These examples show the potential of the method for investigating the kinetics of new drugs in man, at a stage following preliminary toxicology but prior to extensive multi-species toxicology.

The ultimate sensitivity of this method is *sub-pico-molar*. Data can be obtained from as little as $0.1 \mu\text{Ci}$ (3.7 kBq) of radioactivity, longer counting periods can be

used and, under favourable conditions, carbon-11 and fluorine-18 labelled compounds can be obtained at much higher specific activities than those used here e.g. up to $2 \mu\text{Ci pmol}^{-1}$ (74 kBq pmol^{-1}) for carbon-11 (Frost *et al.*, 1990) and $4 \mu\text{Ci pmol}^{-1}$ ($152 \text{ kBq pmol}^{-1}$) for fluorine-18 (Brodack *et al.*, 1986). Such sensitivity for mass would depend on continuing to use a configuration of large detectors to collect counts from large regions with high absolute efficiency. An exciting possibility is that the sensitivity of the method might enable the distribution of highly potent compounds, such as the vasoactive peptides, to be studied for the first time in man.

Another area of potential interest would be to test new drugs, at pharmacologically active doses, against established PET radioligands for receptors and enzymes, so yielding information about site of action, effective dosage and duration of target site occupancy in man. Precedents for the feasibility of these types of study already exist in PET (see for example Farde *et al.*, 1986; Price *et al.*, 1990). Also new drugs might be tested against PET tracers of metabolic pathways to assess the multi-organ metabolic effects of drugs.

The method reported here provides data in a form similar to that initially sought by developers of new drugs, namely timed assays of drug content in particular tissues *ex vivo*. The ability, demonstrated here, to record pharmacokinetics from representative tissues of the human body suggests an opportunity for formulating more advanced kinetic models of the fate of a drug than hitherto possible by administering a drug at the high concentration necessary for assay *in vitro*.

A major limitation on the interpretation of the time course of radioactivity recorded in an organ is that metabolic transfer of the label cannot be identified; the method measures only radioactivity and does not allow to differentiate between parent drug and metabolites. The use of selective enzyme inhibitors could help to resolve such difficulties, as evidenced by the observation that the method is clearly capable of revealing the effect of carbi-DOPA on the gross distribution of L-6-[^{18}F]-fluoro-DOPA and its labelled metabolites (Figure 5).

Studies using drug labelled at varying sites in the molecule would also assist in elucidating metabolic effects.

To date the method has been used to follow regional kinetics up to 2 h after administration. For a rigorous characterisation of the elimination kinetics of drug one needs to quantify concentrations over a time period equating to at least three elimination half-lives of the drug. It is estimated, that by optimising the electronics associated with the detectors, a dynamic range of 1000 would be possible, while still operating within the picomolar concentration range for the drug. This would mean that carbon-11 labelled compounds could be followed for up to 3.3 h and fluorine-18 labelled compounds for up to 20 h, after administration.

In this study the large scintillation probes were uncollimated, so measurements could not be localised to a small area. Scope exists for coincidence counting and for collimating the detectors to give improved spatial resolution, though at some expense to sensitivity. The scintillation detectors have an energy resolution of approximately 8%. This has the advantage that scattered γ -rays only make a small contribution to the peak integral. Furthermore, the system provides scope to improve quantitation by normalising the regional uptake of tracer to small amounts of simple reference tracers. For example, bromine-77 ($t_{1/2} = 57$ h; $\gamma = 239$ keV, 30% and 521 keV, 24%) and potassium-43 ($t_{1/2} = 22.2$ h; $\gamma = 373$ keV, 88% and 618 keV, 81%) may be administered prior to the study to delineate extracellular or intracellular space, respectively (Skrabal *et al.*, 1970). The main γ -ray lines emitted by these reference materials could be clearly

distinguished from the 511 keV photons arising from the decay of carbon-11 or fluorine-18. This would allow drug content to be normalised to either the extracellular or intracellular spaces of the tissue. These data and the envisaged applications of the method are expected to provide a stimulus to industry to develop commercial detector systems which offer better focal delineation without compromising overall sensitivity.

This method depends on a specialised area of radiochemistry in preparing the labelled drug. However, a large number of labelling techniques has now been developed whereby a high proportion of drugs can now be labelled with carbon-11 or fluorine-18, often at a position of choice (see Fowler & Wolf, 1986, Pike, 1988). Such labelling techniques are capable of delivering tens of millicuries of labelled drug in an assured (see, for example, Pike *et al.*, 1990) radiochemically and chemically pure state and at high specific activity. In principle, such activities would provide the opportunity to transport carbon-11 or fluorine-18 labelled drugs considerable distances from the location of radiosynthesis, so enabling kinetic studies in man to be performed widely by methods akin to that described here. Thus, in conclusion, this technique is not only useful in conjunction with a PET programme, but might also evolve to be of great benefit to drug discovery programmes and to the optimisation of ensuing drug therapies.

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