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Using ^{14}C radiolabeled clinical studies to explore and evaluate factors influencing oral exposure.

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INTRODUCTION

Administration of ^{14}C labelled drug product in clinical drug development is usually associated with regulatory ADME studies. However, the increase in sensitivity of bioanalytical detection techniques such as accelerator mass spectrometry (AMS) to measure microtracer amounts of ^{14}C from clinical samples, opens up the opportunity for more innovative study designs in clinical development to increase the understanding of the absorption and bioavailability of drugs. This information can then be used to inform and guide formulation development, support physiologically based pharmacokinetic (PBPK) modelling, and influence program strategy.

Intravenous (IV) microtracer dosing is an established tool for absolute bioavailability assessment. It involves the concomitant administration of an IV microdose of a drug, labelled with microtracer amounts (not more than (NMT) 37kBq (1000 nCi) of ^{14}C, with an oral therapeutic dose of the drug. The IV dose is administered via a short infusion, which terminates at the Tmax of the oral drug. This avoids the concerns of dose dependent kinetics when extrapolating IV PK from a microdose, as the systemic exposure is at therapeutic concentrations. This allows generation of absolute bioavailability without the need of a conventional IV formulation or an IV toxicity safety package, or local tolerability studies as long as pharmacopoeial excipients are used. In addition, dosimetry data and approval for administration of radiolabelled drug products are not required for microtracers, given the low levels of activity administered ($\leq$ 37 kBq).

The following poster describes a 2 part study combing an IV microtracer study (Part 1) with a conventional oral ADME study (Part 2), which can generate definitive IV pharmacokinetic (PK) parameters, absolute bioavailability (F) and information to enable understanding of the factors that influence systemic exposure following oral dosing, in addition to generating regulatory ADME data required for a new drug application. Data from an anonymised example case study is presented to illustrate this approach.

STUDY DESIGN

This was a single centre, open-label, non-randomised, 2-part -part cross over study in 6 healthy male subjects. Subjects were screened for eligibility to participate in the study within 28 days before dosing. Approvals were obtained from Ethics, Medicines and Healthcare products Regulatory Agency prior to subject recruitment. A formal dosimetry report was produced by Public Health England approving the maximum radioactive dose allowable within the ICRP (1992) Category IIa limit of 1mSv. The study outline is described in Figure 1.

The primary objectives were:

- To determine the absolute bioavailability of Drug X in healthy subjects
- To assess the absorption, metabolism and excretion of Drug X after oral administration of [^{14}C]-Drug X to healthy subjects

Figure 1

Part 1 Schedule	Part 2 Schedule
Screening **Day -28 to Day -2**	**Screening** **Day -28 to Day -2**
↓	↓
Admission: Day -1	**Admission: Day -1**
↓	↓
Day 1 **Regimen A:** a single oral therapeutic dose of Drug X tablet followed by **Regimen B:** an IV microdose of ^{14}C-Drug X containing NMT 37.0 kBq [1000 nCi] ^{14}C administered as a 15 min infusion ending at the Tmax of the oral dose	**Day 1** **Regimen C:** a single oral therapeutic dose of ^{14}C-Drug X containing NMT 3 MBq [81 µCi] ^{14}C, administered as a solution
↓	↓
Discharge: Day 3	**Discharge Day 11**

Part 1

- Plasma samples were collected to analyse parent drug via HPLC MS/MS (oral dose), and for total radioactive dose (TRA) and ^{14}C-parent via HPLC AMS (IV dose).
- Samples for haematology and clinical chemistry safety assessments, were also taken at specified time points.

Part 2

- Plasma samples were collected for analysis of parent drug via HPLC MS/MS, TRA via liquid scintillation counting (LSC), and for metabolite profiling and identification.
- TRA in selected blood samples was also measured.
- Excreta were collected to estimate the percentage recovery of TRA in urine and faeces.
- Samples for haematology and clinical chemistry safety assessments, were also taken at specified time points.

RESULTS

Drug X was well tolerated. There were no deaths, severe AEs or serious AEs reported during the study, and no subject was withdrawn as a result of an AE. There were no clinically significant findings in any laboratory assessments, vital signs, ECGs or physical examinations.

Table 1 presents a summary of parameters for Drug X that were estimated from the data generated in the study. IV Clearance of the drug was moderate (48.0 % liver blood flow), and absolute bioavailability was 5.7 % and 6.9 % for the capsule and solution formulation, respectively. The metabolite load following oral and IV administration of ^{14}C Drug X was 98.5 % and 12.1 %, respectively.

Table 2 presents a summary of the mass balance results of Drug X. Average mass balance reached by 264 h post dose was 90 %, with 62.5 % recovered urine and 27.2 % recovered in faeces. A majority of the radioactivity in urine was eliminated by 72 h post dose.

METHODS

The following parameters can be obtained from the data generated in the study;

Part 1:

- Absolute bioavailability (F)

$$F = (AUC_{oral}/AUC_{IV}) \times (Dose_{IV}/Dose_{oral}) \times 100\ \%\ \text{- Equation 1;}$$

where AUC_{oral} is the area under the plasma concentration time curve of drug following oral dosing and AUC_{IV} is the area under the plasma concentration time curve of [^{14}C]-drug following IV dosing

- Metabolite load following IV dosing (ML_{IV})

$$ML_{IV} = (AUC_{TRA,IV} - AUC_{IV})/AUC_{TRA,IV} - \text{Equation 2;}$$

where $AUC_{TRA,IV}$ is the area under the plasma concentration time curve of TRA following IV dosing.

- ($F_{abs} \times F_g$) i.e. the amount of drug appearing in the hepatic portal vein following oral dosing,

$$F_{abs} \times F_g = F/(1 - E_H) - \text{Equation 3;}$$

where F_{abs} is fraction of drug crossing into gastrointestinal epithelial cells, F_g the fraction of drug surviving gut metabolism, E_H is hepatic extraction ratio calculated by Cl_{IV}/hepatic blood flow*, and Cl_{IV} is the IV clearance (assuming clearance is primarily hepatic, metabolic). *Hepatic blood flow is 1450 ml/min[1].

Part 2:

- Metabolite load following oral dosing (ML_{oral})

$$ML_{oral} = (AUC_{TRA,oral} - AUC_{oral})/AUC_{TRA,oral} - \text{Equation 4;}$$

where $AUC_{TRA,oral}$ is the area under the plasma concentration time curve of TRA following oral dosing and AUC_{oral} is the area under the plasma concentration time curve of drug following oral dosing.

Combining data from Parts 1 and 2 of the study enables assessment of F_g

$$F_g = 1 - (ML_{oral} - ML_{IV}) - \text{Equation 5}$$

F_{abs} can be calculated via rearrangement of Equation 3

Table 1

Regimen Route Analyte No. of Subjects	A Oral Drug X N = 6	B IV ^{14}C-Drug X N = 6	C Oral Drug X N = 6
F (%)	5.7	NA	6.9
Metabolite load	NA	0.121	0.985
Hepatic Extraction ratio	NA	0.480	NA
$F_{abs} \times F_g$	NC	NA	0.133
F_g	NC	NA	0.136
F_{abs}	NC	NA	0.978

NA: not applicable; NC: not calculated

Table 2

Matrix	Collection Time (h)	Mean Cumulative %Ae [%] (% CV)
Urine	0-168	62.5 (4.8)
Faeces	0-264	27.2 (8.2)
Total	0-264	89.7 (4.3)

DISCUSSION

Data from the IV microtracer limb of the study showed that the Cl_{IV} of the drug was moderate (E_H = 0.480), although the oral bioavailability of Drug X was low, 5.7 % and 6.9 % for the capsule and solution formulation, respectively. Elucidating whether the poor bioavailability was due to high gut metabolism or poor absorption (e.g. poor Fabs) would provide the Sponsor with valuable information as to whether it would be possible to improve oral bioavailability via a formulation strategy. Examination of the metabolite load following IV and oral administration of ^{14}C labelled Drug in Part 1 and Part 2 respectively, revealed that there was a very high metabolite load orally (98.5 %), i.e. parent drug accounted for only 1.5 % of circulating drug related material. However, following IV dosing the metabolite load was substantially lower at 12.1 %. The difference between these 2 estimates gives a measure of the fraction of drug surviving gut metabolism, which in this case was low (13.6 %). Thus, the low bioavailability can be attributed to high gut metabolism rather than poor absorption (F_{abs} = 97.8 %). This information enables the client to consider formulation strategies to overcome the gut metabolism e.g. a modified release formulation which may deliver the drug further down the gastrointestinal tract where metabolism due to CYP3A4 is less, due to lower enzyme expression. The client has also generated invaluable information for use within mechanistic PBPK models.

CONCLUSIONS

- The study design presented successfully enables calculation of definitive IV PK parameters and absolute bioavailability, as well as generating regulatory ADME data.
- The study design enabled estimation of parameters that influence systemic exposure e.g. F_{abs}, F_g, E_H, and an understanding of the cause of poor bioavailability.
- This information can be utilised to inform and guide formulation development, support PBPK modelling approaches and influence program strategy in clinical development.

REFERENCES

1. Davis, B and Morris, T. Physiological Parameters in Laboratory Animals and Humans. Pharmaceutical Research Vol 10. No. 7. 1093-1095. 1993.