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Simultaneous oral therapeutic and intravenous ^{14}C-microdoses to determine the absolute oral bioavailability of saxagliptin and dapagliflozin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Determination of absolute bioavailability may be useful in early drug development and this assessment is acquiring new importance for regulatory filings of new medicines. Simultaneous dosing of a therapeutic oral dose with an intravenous ^{14}C-microdose, and detection with liquid chromatography-tandem mass spectrometry (LC-MS/MS) and accelerator mass spectrometry (AMS), respectively, has not been widely used for determining the absolute bioavailability of new chemical entities due to uncertainties surrounding AMS accuracy and health authority views on this approach.

WHAT THIS STUDY ADDS

- Using a simultaneous intravenous ^{14}C-microdose given at the t_{max} of an orally administered unlabelled drug, and analysis using AMS detection, is a resource-saving and viable approach to determine the absolute bioavailability of a new chemical entity, provided a robust AMS method is used. The absolute bioavailability values of two new diabetes medicines, saxagliptin and dapagliflozin, were determined using this approach to characterize fully their pharmacokinetics. These studies were used in the regulatory submissions of these drugs.

AIM

To determine the absolute oral bioavailability ($F_{p.o.}$) of saxagliptin and dapagliflozin using simultaneous intravenous ^{14}C-microdose/therapeutic oral dosing (i.v.micro + oraltherap).

METHODS

The $F_{p.o.}$ values of saxagliptin and dapagliflozin were determined in healthy subjects (n = 7 and 8, respectively) following the concomitant administration of single i.v. micro doses with unlabelled oraltherap doses. Accelerator mass spectrometry and liquid chromatography-tandem mass spectrometry were used to quantify the labelled and unlabelled drug, respectively.

RESULTS

The geometric mean point estimates (90% confidence interval) $F_{p.o.}$ values for saxagliptin and dapagliflozin were 50% (48, 53%) and 78% (73, 83%), respectively. The i.v.micro had similar pharmacokinetics to oraltherap.

CONCLUSIONS

Simultaneous i.v.micro + oraltherap dosing is a valuable tool to assess human absolute bioavailability.

Introduction

During the clinical development of new medicines intended for non-i.v. clinical use, the determination of the absolute oral bioavailability ($F_{p.o.}$) can be useful in identifying characteristics that may present developmental challenges, e.g. absorption and/or first-pass effects [1]. Major health authorities have indicated that the determination of $F_{p.o.}$ is a recommended [2, 3] or mandatory [4] requirement in the evaluation of a candidate drug's biopharmaceutic properties and the establishment of the clinical pharmacology profile.

Traditionally, the $F_{p.o.}$ of a compound administered via a non-i.v. route is determined using a crossover study design using either clinically relevant doses or doses that provide sufficient concentrations to enable adequate charaterization of the pharmacokinetic profile. This approach ensures that the pharmacokinetics via each route are comparable at therapeutically relevant systemic concentrations, but it has a number of challenges. These include the development of a stable and sterile intravenous (i.v.) liquid formulation and the requirement for i.v. animal toxicology studies for risk assessment associated with exposing healthy subjects to a new drug via a route not previously studied [5]. An alternative approach that simultaneously addresses the above issues is to utilize radiolabelled or stablelabelled microdoses for the i.v. component of the study. By using very low doses (typically 1/100th to 1/1000th of the therapeutic dose and $\leq$100 μg), a liquid formulation for i.v. administration can be compounded extemporaneously from drug powder and an appropriate vehicle at the clinical site. Furthermore, the need for i.v. toxicology assessments are reduced or not needed if high dose oral animal toxicology data are available [5]. Lastly, since the delivered dose is very low, this approach is very useful for drugs with poor aqueous solubility where the delivery of therapeutic doses in a traditional i.v. formulation may not have been feasible.

Several investigators have raised concerns about possible non-linearity of pharmacokinetics following a microdose relative to the extravascular therapeutic dose and challenges included the development of a bioanalytical assay with sufficient sensitivity to characterize the low plasma concentrations [6, 7]. These concerns may have limited the acceptance of the microdose approach in drug development since the receptiveness of regulators to this methodology was uncertain. This probably resulted in conservatism amongst drug developers to employ non-traditional approaches to $F_{p.o.}$ determination. As has been demonstrated in several studies on marketed drugs [8–10], the linearity and bioanalytical sensitivity issues can be simultaneously addressed by administering the i.v. microdose ([^{14}C]-labelled compound) at the time of maximum concentration (t_{max}) of the extravascular (unlabelled) therapeutic dose. This innovative approach exposes the body to therapeutically relevant concentrations of the compound, addresses concerns regarding pharmacokinetic linearity and improves precision of the $F_{p.o.}$ estimate due to the lack of inter-occasion variability. If the administered radioactivity is kept very low (e.g. <250 nCi), subjects will be exposed to extremely low amounts of radiation and therefore, supporting data such as a dosimetry study in animals may not be required [5]. In order to achieve sufficient bioanalytical sensitivity for the microdose and differentiate the plasma concentrations resulting from the radiolabelled dose from the oral dose, the ultra-sensitive method of accelerator mass spectrometry (AMS) can be used to quantify plasma concentrations of radiolabelled parent drug derived from the i.v. route based on measurement of ^{14}C [11].

The studies reported here describe the determination of the $F_{p.o.}$ of two novel oral agents for type 2 diabetes mellitus, saxagliptin (Onglyza™), a dipeptidyl peptidase-4 inhibitor (DPP4), and dapagliflozin, a sodium glucose co-transporter-2 (SGLT2) inhibitor, using a simultaneous oral therapeutic dose and i.v. ^{14}C-microdose administration paired with fully validated bioanalytical methods.

Methods

Clinical study designs

The study protocols were approved by the local ethics committees and the studies were conducted according to the principles of Good Clinical Practice. The healthy subjects provided their written informed consent to participate in these studies. The subjects in both studies were all white males, ranging in age from 18 to 45 years with BMI values ranging from 19.2–30.2 kg m^{-2}.

The i.v. dose in both studies was administered into one arm of the subject and the contralateral arm was used for pharmacokinetic sampling and clinical laboratory blood draws. Blood samples were obtained to investigate the pharmacokinetics of unlabelled compounds (measured by LC-MS/MS) and [^{14}C]-saxagliptin and dapagliflozin (using AMS) over a 24 or 49 h period, respectively, following the oral dose. The actual i.v. dose administered to each individual was calculated based on the volume of solution delivered in both studies. Physical examinations, vital sign measurements, ECG and clinical laboratory evaluations were performed, and subjects were closely monitored for adverse events at selected times throughout the studies. The subjects were discharged on day 2 (saxagliptin) or day 3 (dapagliflozin).

Absolute bioavailability study with saxagliptin

Following an overnight fast of at least 10 h, subjects (n = 8) received a single oral dose of 5 mg saxagliptin (Onglyza™ tablets) followed by a dose of approximately 40 μg of [^{14}C]-saxagliptin solution (5 ml of a 10 μg ml^{-1} containing <270 nCi in isotonic buffer manufactured by Quotient Bioresearch Limited) infused i.v. over a 15 min period beginning 1 h after the oral dose (i.e. at t_{max} of the oral dose). The [^{14}C]-saxagliptin had a radiochemical purity of

99.2%, a specific activity of 5.19 μCi mg^{-1} and the saxagliptin in the i.v. solution formulation consisted of 2.6% [^{14}C]-saxagliptin and 97.6% unlabelled saxagliptin. The [^{14}C]-saxagliptin and unlabelled saxagliptin were manufactured under Good Manufacturing Practice conditions by Bristol-Myers Squibb.

Absolute bioavailability study with dapagliflozin

Following an overnight fast of at least 10 h, subjects (n = 7) received a 10 mg oral dose of dapagliflozin followed 1 h later (i.e. at t_{max} of the oral dose) by an approximately 80 μg dose of [^{14}C]-dapagliflozin solution (0.32 ml of a 250 μg ml^{-1} containing ~200 nCi in isotonic buffer manufactured by Bristol-Myers Squibb) infused i.v. over a 1 min period. The [^{14}C]-dapagliflozin had a radiochemical purity of 99.3%, a specific activity of 2.02 μCi mg^{-1} and the dapagliflozin in the i.v. solution consisted of 1.6% [^{14}C]-dapagliflozin and 98.6% unlabelled dapagliflozin. The [^{14}C]-dapagliflozin and unlabelled dapagliflozin were manufactured under Good Manufacturing Practice conditions by Bristol-Myers Squibb.

Bioanalysis

The concentrations of [^{14}C]-saxagliptin and [^{14}C]-dapagliflozin in human plasma following the i.v. doses were assayed by AMS following sample preparation using protein precipitation, LC-fractionation and sample graphitization. The LLOQ of the assay was determined to be the concentration where the presence of saxagliptin and dapagliflozin could be reliably differentiated from the background ^{14}C observed in the pre-dose study samples and the limit of detection of the AMS. The AMS methods were validated for accuracy, precision and selectivity prior to the analysis of study samples. The validation for the dapagliflozin did not use a standard curve. However, a technology-based and scientifically sound validation was performed which included accuracy, precision and selectivity assessments of QCs. The lower limit of quantitation (LLOQ) of the dapagliflozin AMS assay was determined to be 9.074 pg ml^{-1}. The validation for the saxagliptin assay, which did use a standard curve, also demonstrated the accuracy, precision, stability, specificity and recovery of the method across the concentration range of 0.025 to 15.0 dpm min^{-1} ml^{-1}, equivalent to 1.91 to 1144 pg ml^{-1} [12]. The results from two accuracy/precision (A/P) runs showed that application of a traditional validation approach which utilized standard plots, quality controls and stability assessments to AMS methods can meet widely accepted bioanalytical criteria for accuracy and precision (±15% in general or ±20% for the LLOQ) [13, 14]. Dilution integrity up to three fold, processed stability up to 384 h and frozen sample in matrix stability up to 52 days at −20°C were also demonstrated through the two A/P runs.

Plasma concentrations of unlabelled saxagliptin [15] and dapagliflozin from human plasma were determined by validated LC-MS/MS assays, in which analytical runs using appropriate calibration curves and QC samples met pre-established acceptance criteria according to current regulatory guidelines [13]. The upper and lower range of quantitation for saxagliptin was from 0.10 to 50 ng ml^{-1} and for dapagliflozin was from 1.0 to 200 ng ml^{-1}. The between- and within-run coefficients of variation for the assays were less than 12%.

Pharmacokinetic analysis

The concentration–time data of saxagliptin and dapagliflozin in plasma were analyzed by standard, non-compartmental methods [16] using the pharmacokinetic software programs WinNonlin software (Ver. 5.0.1; Pharsight, Mountain View, CA, USA) or Kinetica (Ver. 4.4.1; Thermo Fisher Scientific, Inc., Waltham, MA, USA), respectively.

Results

Concentration-time profiles of [^{14}C]-saxagliptin and [^{14}C]-dapagliflozin and the corresponding non-labelled concentrations in plasma are shown in Figure 1A and B without dose normalization to illustrate the ~200-fold difference in systemic drug concentrations of the labelled and unlabelled analytes. The pharmacokinetic parameter values are presented in Table 1.

The geometric mean for the point estimates of $F_{p.o.}$ (90% confidence intervals) for saxagliptin and dapagliflozin were 50% (48, 53%) and 78% (73, 83%), respectively. The arithmetic mean half-life values for the i.v. and oral doses (arithmetic mean ± SD half-life values for saxagliptin: 7.5 ± 0.6 and 5.7 ± 0.4 h, respectively; dapagliflozin: 12.2 ± 5.3 and 13.7 ± 3.4 h, respectively) were similar and the plasma concentration–time terminal elimination phases for each route were parallel. The volumes of distribution following i.v. administration for both drugs were greater than total body water and body weight showing that they both distributed out of the plasma compartment. The total blood systemic clearance of both drugs (i.e. total plasma clearance corrected for the distribution between whole blood and plasma: 0.62 for saxagliptin and 0.88 for dapagliflozin) following i.v. administration was substantially less than the blood flow to the liver and the kidney combined [17].

Discussion

The $F_{p.o.}$ values obtained for both saxagliptin and dapagliflozin (50 and 78%, respectively) are consistent with their individual mass balance/absorption, distribution, metabolism and elimination (ADME) study findings [18–20]. The radioactivity recovered in urine of an oral radiolabelled dose in ADME studies is reflective of the minimum fraction of the dose that is absorbed from the gastrointestinal tract. For both drugs, ~75% of the administered dose was

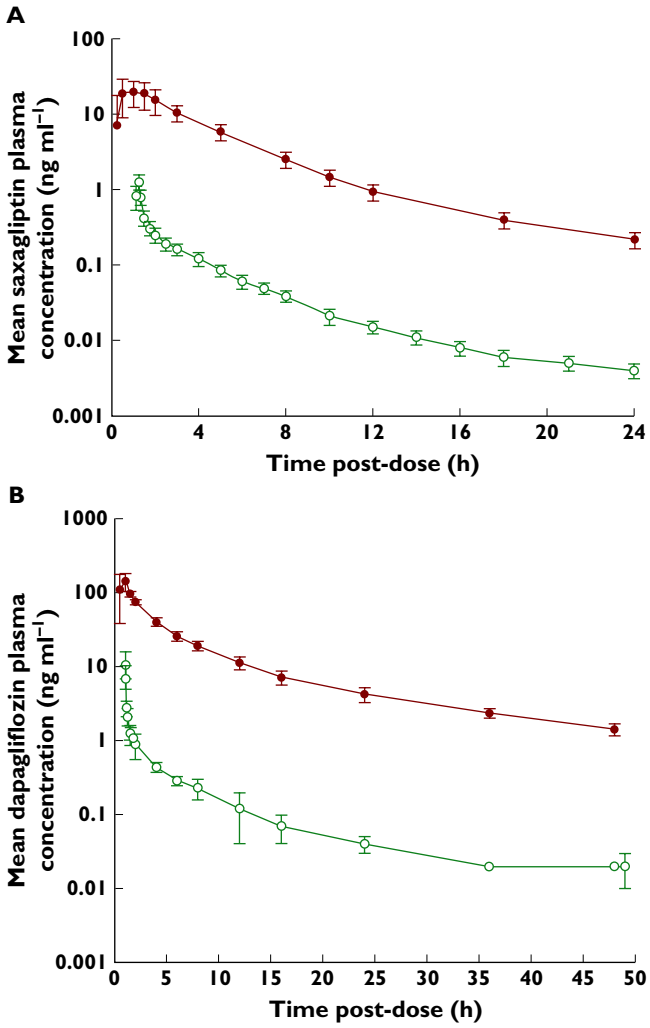

Figure 1

Mean ± SD plasma concentration–time profiles of (A) [^{14}C]-saxagliptin (—○—) and non-labelled saxagliptin (—●—), (n = 8) and (B) [^{14}C]-dapagliflozin (—○—) and non-labelled dapagliflozin (—●—), (n = 7) in healthy subjects administered 1 h after the oral dose

recovered in the urine as mixtures of parent and metabolites. Saxagliptin is a CYP3A4/5 substrate [20] and, based on the high activity of this enzyme system in the gut and liver [21], is likely to be subject to higher extent of first-pass metabolism than dapagliflozin, which is metabolized primarily by glucuronidation [22].

Similar half-life values for the i.v. and oral doses of saxagliptin or dapagliflozin and their respective parallel plasma concentration–time terminal elimination phases implies that the i.v. microdose behaves in a kinetically similar manner to the oral therapeutic dose. Although the half-life values appear to be somewhat different after i.v. and oral saxagliptin administration, this difference was attributed to the different sampling schemes used during the elimination phase when half-life was estimated. Using the same time points for i.v. and oral saxagliptin, the calculated half-life values were 5.9 h and 5.7 h, respectively. These results show that microdoses administered at the t_{max} of the extravascular dose are not subject to non-linear pharmacokinetics as might be the case in crossover studies and that the microdose approach allows an accurate and precise determination of $F_{p.o.}$ at therapeutically relevant concentrations in a single period study. Finally, the simultaneous administration of oral and i.v. ^{14}C-microdoses for the determination of $F_{p.o.}$ has several resource and time saving advantages over traditional study designs. The microdose approach is simple (one period), i.v. formulation development is significantly abbreviated and prerequisite i.v. toxicology studies can be avoided.

These pioneer studies represent the first $F_{p.o.}$ studies using the ^{14}C-microdose design that have been utilized in support of the regulatory review of new medicines. These studies serve as models for $F_{p.o.}$ assessments using simultaneous oral/i.v. microdosing. For saxagliptin and dapagliflozin major health authorities have accepted this approach and the method offers potential savings in both resources and time in the drug development process.

Table 1

Summary statistics for plasma pharmacokinetic parameters of saxagliptin and dapagliflozin following oral administration and [^{14}C]-0saxagliptin and [^{14}C]-dapagliflozin following i.v. administration to healthy subjects

Pharmacokinetic parameter	Oral 5 mg saxagliptin (n = 8)	40 μg i.v. [^{14}C]-saxagliptin (n = 8)	Oral 10 mg dapagliflozin (n = 7)	80 μg i.v. [^{14}C]-dapagliflozin (n = 7)
C_{max} (ng ml^{-1})	23.8 (31)	1.2 (29)	143 (29)	10.2 (49)
t_{max} (h)	0.81 (0.5, 1.5)	0.26 (0.25,0.33)	1.03 (0.50,1.50)	0.03 (0.03,0.08)
AUC(0,∞) (ng ml^{-1} h)	84.4 (24)	1.3 (19)	628 (17)	6.8 (20)
$t_{1/2}$ (h)	5.7 (0.43)	7.5 (0.60)	13.7 (3.44)	12.2 (5.25)
V_{ss} (l)	NA	123 (30)	NC	118 (32)
CL (ml min^{-1})	NA	495 (19)	NC	207 (23)
$F_{p.o.}$ (%)	50 (48, 53)	NA	78 (73, 83)	NA

AUC(0,∞), area under the plasma concentration–time curve extrapolated to infinity; CL, total systemic clearance; C_{max}, maximum observed plasma concentration; %CV, percent coefficient of variation; $F_{p.o.}$ = absolute oral bioavailability; NA, not applicable; NC, not calculated; t_{max}, time of C_{max}. For $t_{1/2}$ and V_{ss}, mean (SD) values are presented. For $F_{p.o.}$, 90% confidence intervals for the geometric mean values are presented. The geometric mean and %CV values are presented for all other parameters.

Competing Interests

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