Pharmacokinetic Aspects of Multiple Dose Studies

Steven G. Woolfrey and James Gilmour Morrison

Contents

Abstract

The development of a new chemical entity (NCE) in man is highly regulated and normally starts with a single ascending dose study (SAD) followed by a multiple ascending dose study (MAD), both in young healthy volunteers using the route and dose regime proposed in the final product. The aim of a MAD study is to establish the safety, maximum tolerated dose, and pharmacokinetics of the NCE on repeated dosing, usually to steady state. This

S. G. Woolfrey (✉)
Independent Pharmaceutical Consultant – Clinical Pharmacology and Pharmacokinetics, Newcastle Upon Tyne, UK
e-mail: Steven.Woolfrey@outlook.com

J. G. Morrison
GW Pharmaceuticals, Cambridge, UK

F. J. Hock, M. R. Gralinski (eds.), *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*,
https://doi.org/10.1007/978-3-319-56637-5_4-1

chapter describes the pharmacokinetic aspects of MAD studies and some of the design modifications that can be included in the study design. The MAD may be one of the few opportunities for assessing the pharmacokinetics of the compound in detail following multiple dosing, albeit not usually in patients. Previous data from the SAD should be used to optimize the design of the MAD including blood sampling regime, lower limit of sensitivity for the bioanalytical assay, and pharmacokinetic analysis. In addition to the pharmacokinetics and safety, the design should consider the need to characterize drug-related material in urine, carry out a preliminary assessment of the metabolic route, and characterize the potential for CYP3A4 induction. Suggestions are also provided for an approach when the impact of prandial status is unknown and more frequent dosing other than once daily is required.

Purpose and Rationale

The clinical development of a new chemical entity (NCE) is highly regulated (ICH–E8 1997), although the approach varies with the type of therapeutic class. Oncology agents are toxic at all dose levels and are normally only tested in oncology patients. Other therapeutic agents may be tested in normal volunteers, and it is these products that are the object of this chapter.

The clinical development of a non-oncology NCE normally begins with a single ascending dose (SAD) study in healthy young male volunteers, aged between 18 and 45 years. As virtually all drugs are administered repeatedly, it is important that the safety and pharmacokinetics are also investigated following multiple dosing. A SAD study is therefore normally followed by a multiple ascending dose (MAD) study, also in healthy young male volunteers. In this, the investigator aims to establish the safety, maximum tolerated dose at steady state, and pharmacokinetics of the NCE before proceeding into patients. This may be one of the few opportunities for assessing the pharmacokinetics of the compound in detail following multiple dosing, albeit not usually in patients. The route and, ideally, the dose regime should be those proposed for the final product at registration. The choice of subjects is usually young healthy male volunteers, but regulatory authorities now recognize the importance of studying females at an early stage of development (Liu and Mager 2016) and encourage their inclusion (ICH 2005).

The aim of this chapter is to describe the process for setting up and assessing the pharmacokinetic components of MAD Phase I studies in man. It is assumed that a SAD study will have already been carried out and data are available from that study. For the purpose of this chapter, it is generally assumed that young healthy male volunteers are used for the assessment, measuring plasma levels of the NCE given by the oral route; however, various design modifications are also considered.

A commonly used approach for the design of MAD studies in healthy volunteers is to administer multiple doses of the NCE to steady state, with detailed pharmacokinetic assessment on Day 1 and at steady state. In order to characterize the approach and time to steady state, pre-dose samples are also taken throughout the dosing period. Typically, this type of study is carried out in small groups of separate subjects per dose group, without consideration of the statistical power.

Any clinical study should always try to maximize data collection in order to justify the use of human volunteers, minimize development time, and justify the overall cost of the study. Consequently, it is usual practice to expand a MAD study to provide a preliminary investigation of metabolism and urinary pharmacokinetics where appropriate and contribute to an overall Phase I assessment of the importance of metabolic polymorphisms. Other subgroups are also possible depending on the properties of the NCE.

Procedure

The protocol and design of a typical MAD study in healthy male volunteers have a fairly standard layout, and the main protocol headings are shown

below. The number of dose groups in a MAD study will vary depending on the nature of the compound and the pharmacokinetics known at the time of the study. The use of three or four ascending dose groups is common practice for a compound with a reasonable safety profile, where predictable pharmacokinetic are expected.

A MAD study is normally carried out immediately after a SAD study. The primary purpose of a MAD study is to characterize the safety, as well as the pharmacokinetics after multiple dosing. The sections below only cover the pharmacokinetic aspects of the MAD study, reflecting the objectives of this work.

Protocol Outline

Objectives

As MAD studies involve an assessment of safety, as well as the pharmacokinetics of the NCE, the protocol has to reflect both objectives. It is normal practice to have safety as the primary objective and the pharmacokinetic characterization as a secondary objective. Investigation of other aspects of the NCE, e.g., preliminary gender effect, may also be stated here as tertiary objectives.

A typical protocol entry for the objectives of a MAD study is given below:

- Primary – To assess the safety and tolerability after repeated oral doses of ABC1234 to steady state
- Secondary – To assess the pharmacokinetic parameters after repeated oral doses of ABC1234 to steady state

Study Design

A variety of designs are possible for a MAD study, but each group needs to involve a small number of placebo subjects for comparison of the safety data. Some plasma samples may be used from the placebo subjects to confirm that the placebo subjects were not dosed with active compound, but otherwise they play no further part in the pharmacokinetic assessment. For example, plasma samples from the placebo subjects might be taken at the anticipated T_{max} and just before the next dose for bioanalysis on each main pharmacokinetic sampling day.

Although statistical analysis is carried out on the safety and pharmacokinetic data, the study is not powered to achieve statistical significance. The overall objective is to use the smallest number of subjects to characterize the multiple dose safety and pharmacokinetics of the NCE.

As the design of a MAD study involves both active and placebo-treated subjects, they are normally run as double-blind designs, involving the generation and use of a subject randomization sheet with the usual precautions and rules for accidental and required unblinding, respectively.

The design preferred by the author is a double-blind design of three or four treatment groups of 12 subjects, nine treated with active, and three with placebo. Subjects are randomly assigned to a group and each group treated repeated with an increasing dose of drug in the selected prandial state to steady state. The likely time to steady state is assessed from the results of the SAD study. The safety and pharmacokinetic data are assessed at each dose level before progressing to the next higher dose.

Inclusion Criteria

Overall inclusion criteria applied in a SAD study should be applied to the MAD study. As with virtually all Phase I studies, the subjects are normally men between 18 and 45 years in good health. Permitted concomitant medications allowed in the SAD study should normally be allowed in the MAD, but for both study types, the list should be very minimal. Any concomitant drugs that could affect the safety and pharmacokinetics of the NCE are normally excluded.

Treatments and Doses

The type of formulation at the time of the SAD and MAD studies is normally experimental hard gelatin capsules. It is recommended that the same formulation is used for the SAD and MAD studies to eliminate any differences due to formulation. If other formulations need to be examined, these should be included as separate subgroups.

The dosing process should be identical for the MAD and SAD studies and between MAD doses. Each daily treatment should be given as a combination of capsules with a standard volume of water (typically 200 ml) in the morning for a once-daily regime. A discussion on the use of fed and fasted prandial state is given in section "Critical Assessment of the Method."

Pharmacokinetic Data

In order to characterize the plasma pharmacokinetics of NCE, frequent blood samples for the measurement of total drug taken over a dosing interval on Day 1 and at the end of the study over the last dosing interval are required. About 12 to 15 samples are normally sufficient to characterize the pharmacokinetic of most NCE. Beyond the end of the last dosing interval, additional samples are taken in order to characterize the terminal half-life at steady state, typically another two to five samples depending on the expected half-life. Blood samples are taken pre-dose (C_{trough}) on selected days during the dosing phase to measure the approach and time to steady state. In all cases, the authors recommend duplicate pharmacokinetic plasma samples, one transported to the site of bioanalysis and the other retained at the clinical site, in case of problems in transport or bioanalysis.

A pre-dose blood sample is normally collected to investigate allelic variants of drug metabolism enzymes and drug transporters as part of the development process as described in section "Genotyping Data."

If the NCE is cleared in urine, then urine should be collected for pharmacokinetic analysis as described in section "Need to Measure Drug in Urine."

Bioanalytical work needs to be carried out to GLP and GCP standards using fully validated assays. Stability studies are needed to support the storage regime for plasma (normally frozen at −20 °C or −70 °C) and collection process from blood.

Current development regulation requires that the pharmacokinetic analysis is carried out using a model-independent approach (Rowland and Tozer 2011). A description of the software, pharmacokinetic parameters, statistical techniques, and quality assurance procedures needs to be included in the protocol. Statistical analysis may be described in detail in the protocol or, more usually, in a separate statistical analysis plan.

The level of detail provided in the protocol for the pharmacokinetic analysis should specify the parameters, their formula, and procedures for assessment, for example, half-life estimates and extrapolations. This is particularly important if the pharmacokinetic analysis is carried out by an independent organization.

Evaluation

The pharmacokinetic analysis of a NCE is carried out using actual blood sampling times on individual plasma concentration-time profiles. Data can only be excluded if there is a fully documented reason indicating a problem with the bioanalysis, dosing, or subject compliance, which must be fully explained and documented in the final study report.

The following pharmacokinetic parameters form the backbone of all MAD studies determined for each individual plasma concentration-time profile:

Day 1 – C_{max}, t_{max}, $AUC_{last,}$ and AUC_{τ}
Day 14 – C_{max}, t_{max}, AUC_{τ} and $t_{1/2}$
C_{trough} plasma concentrations on Days 2–14

Other parameters may be needed as outlined in section "Example."

Assessment of dose proportionality is an important objective of the MAD study. One approach is to assess dose proportionality for C_{max} and AUC_{τ} on Day 1 and at steady state separately, using the empirical power model (Parameter $= \alpha \times \text{dose}^{\beta}$), along with an "estimation" interpretation, according to the recommendations of Gough et al. (1995). Within-subject and total standard deviations for $\log(C_{max})$ and $\log(AUC_{\tau})$ are estimated.

Accumulation of the NCE is also assessed in the MAD study using the Day 1 and steady-state

data for C_{max} and AUC_{τ} (R_{Cmax} and R_{ac}). Accumulation is examined statistically for log (ratio of day steady state/Day 1) C_{max} and AUC_{τ}, with a linear fixed effects model. Accumulation ratios are assessed for each dose level separately, as well as pooled across dose levels within the fixed effects model framework.

The occurrence of steady state is typically assessed by fitting C_{trough} values to a nonlinear mixed effects model in order to predict the time to achieve 90% of the steady-state trough concentration, taking into account any dose differences.

Half-life determined at steady state is compared across the doses using a linear mixed effect model.

Summary statistics are calculated for all parameters and the data then listed on an individual basis and with the summary statistics. The data may be plotted to show both the individual and mean values so that trends can be easily identified and investigated. Reporting is carried out according to ICH guidelines (ICH-E3 1995).

Critical Assessment of the Method

Use of Previous Data to Design Study

In order to optimize the sampling regime and choose the doses for the MAD study, it is recommended that data from a clinical SAD study are used to predict the pharmacokinetics on multiple dosing, including accumulation, time to steady state, and exposure at steady state. Such modeling and simulation will help provide confidence that the likely steady-state exposures will be within safe limits, but are adequate to allow a pharmacological effect in Phase II. It will also provide confidence that the bioanalytical assay has sufficient sensitivity to characterize the pharmacokinetics on all sampling days.

Preliminary Pharmacokinetic Analysis

It is common practice to carry out an interim pharmacokinetic analysis for each dose of the MAD study to check exposure levels and the pharmacokinetics prior to progression to the next dose. As the bioanalysis can take a couple of days to turn around, many investigators use a "N-1" approach to assess each dose: Typically this preliminary analysis uses nominal blood sampling times and bioanalytical data that has not been subjected to a full quality control check, on the basis that it will be repeated in the final analysis using actual sampling time data and final analytical data. The preliminary pharmacokinetics of the previous dose is examined at the same time as the safety data from the current dose in order to make a decision on whether it is feasible to proceed to the next dose in the dose escalation. In order to carry out the interim analysis in a timely manner, close coordination is required between sample transfer to the site of bioanalysis, the bioanalysis itself, and the pharmacokinetic analysis.

Approach to a Double-Blinded Study

Multiple dose Phase I studies are normally carried out in a double-blinded manner in order to aid the interpretation of the safety data. The bioanalyst and pharmacokineticist, however, effectively "unblind" the study in their interim analyses. It should be remembered that often the bioanalysis cannot wait until the completion and "lock" of the safety data, particularly if preliminary pharmacokinetic data have to be released prior to the next dose increase. Pragmatic procedures, therefore, need to be written into the protocol to protect the safety assessment from accidental "unblinding," which, for example, can be relevant if adverse events are recorded for individual subjects. Pragmatic approaches to interim pharmacokinetic data include removing subject identifiers for individual data and only presenting summary data, e.g., mean and SD.

Dosing in Fed or Fasted State

The effect of the prandial state on the pharmacokinetics of the NCE may be unknown at the point of carrying out a MAD study. If it has been examined prior to the MAD study, then the prandial

state that significantly maximizes the exposure (typically greater than twofold) is recommended, as this will probably be recommended for the final product. If data are unavailable or previous data have shown no major influence of food, then dosing in the MAD study should be carried out after an overnight fast, with a light breakfast allowed 2 h after dosing. The same prandial state for dosing should be used throughout the MAD.

Blood Sampling

The blood sampling regime is fundamental to the successful outcome of any pharmacokinetic assessment. Great care must, therefore, be taken to ensure that an adequate number of samples, appropriately placed during the dosing regime, are built into the design that yield levels above the lower limit of detection of the assay. The data from the SAD study and predictions of the pharmacokinetics on multiple doing should be used to help decide on the blood sampling regime and the target assay sensitivity.

Practical considerations also need to be considered with respect to the actual timing of blood samples: Sampling during the night when the subjects are normally asleep is possible but should obviously be kept to a minimum and avoided if at all possible.

Genotyping Data

The pharmacokinetics of some drugs can be affected by genetic polymorphisms of drug metabolism enzymes, which may lead to an apparent high level of pharmacokinetic variability and, in extreme cases, safety concerns. By the time an NCE enters clinical development, some knowledge is available on the likely impact of the major genetic polymorphisms from in vitro data. As a result, subjects should be screened for the major or compound-specific metabolic genetic polymorphisms, in order to assess the effect of these on the pharmacokinetics of the NCE at the end of the Phase I program. At this point data will be available from a limited number of subjects showing a number of allelic variants of metabolism enzymes.

Modification of the Method

Additional Pharmacokinetic Parameters After Oral Dosing

There are many pharmacokinetic parameters that can be calculated to characterize the pharmacokinetics of a compound after multiple oral dosing, and the most common used for summarizing the data are those given in section "Evaluation."

Three optional parameters that are often calculated, include the apparent volume of distribution, clearance, and mean residence time, all estimated at steady state in MAD studies. It must be remembered that the apparent volume of distribution and clearance is influenced by the bioavailability of the compound, which may well be unknown early in clinical development.

There are a variety of apparent volumes of distribution terms that may be calculated. It is generally regarded, however, that the volume of distribution at steady state (V_{ss}/F) is the most robust (ICH-E3 1995), although the apparent volume term for the terminal phase (V_z/F) is the one usually calculated.

Other parameters that are sometimes stated include C_{min}, $C_{average}$ (at steady state), t_{last}, and $t_{1/2\ effective}$, the latter being the half-life estimated from the time to achieve steady state.

In some cases, there may be a lag in the absorption of a compound, possibly due to the dispersion of an oral dose form. Such a delay is usually observed in the SAD study, and it may be important to include t_{lag} in the MAD study. If a lag time is not observed after a single dose, it is probably unnecessary to measure this parameter in the MAD study, unless there is a significant change in dosage formulation.

Impact of Dosing More Frequently Than Once Daily

In some cases, a NCE is administered more frequently than once daily, in order to achieve adequate exposure during the dosing interval. The principles that have been developed for multiple dosing assuming once-a-day dosing also apply to the situation when dosing is more frequent. In essence, the pharmacokinetic analysis is carried per dosing interval.

With once-daily dosing, the impact of diurnal variation on exposure is usually not apparent. With more frequent dosing (e.g., twice daily), however, diurnal variation may have a significant effect on exposure and, ultimately, the therapeutic effect. This can be assessed by characterizing the pharmacokinetics at steady state under two dose periods, these being carefully chosen to maximize the likely differences (e.g., one during the day and one at night). When setting up such comparisons, the number of blood samples that can reasonably be taken may become problematical either because of their number and volume of blood or because of the need to sample intensively during the night. Care needs to be taken to make sure that the exposure data will be comparable between the two dosing periods and other Phase I studies, e.g., SAD.

When developing a drug, the final therapeutic dose is highly influenced by the exposure ratio between the proposed human dose and the no observed adverse effect level (NOAEL) or no effect level (NOEL) in the most sensitive animal toxicology species. The animal data are often, but not always, generated by once-daily dosing and so a strategy needs to be established beforehand on how the animal/human exposure comparisons will be determined.

Need to Measure Drug in Urine

The need to sample urine for the assessment of the NCE will depend whether the compound is eliminated via the kidneys to any great extent. This is often assessed in the single ascending dose study; however, it may at this very early stage be based on preliminary non-validated assays. It is recommended that if >5% of the dose is renally excreted as parent drug, then the quantification of drug in urine via a fully validated assay needs to be carefully considered, as part of the MAD study.

There are a variety of ways to characterize the urinary pharmacokinetics of a NCE. It is recommended, however, that a urine collection is made over a dosing interval at steady state from which the following parameters can be calculated using standard pharmacokinetic principles (Rowland and Tozer 2011):

- Amount excreted under a dosing interval ($Ae_{0\text{-}T}$)
- Fraction of the dose excreted per dosing interval ($Fe_{0\text{-}T}$)
- Urinary Clearance ($Cl_{R0\text{-}T}$)

Care needs to be taken to ensure a complete collection of urine (as the volume of urine/dosing period is critical to the assessment) and that the urinary collection and storage process maintain the integrity of the compound. It is usual to collect the urine over various collection periods within a dosing interval from which aliquots for bioanalysis can be frozen and sent for analysis. As for plasma, duplicate samples for bioanalysis are recommended, one being maintained at the site as a backup.

Characterization of Metabolites

The development of any NCE requires the characterization of its route of metabolism and excretion in man (ICH E8). In the past, this was done in a small group of human subjects using a radiolabeled NCE, usually with ^{14}C, the study often carried out early in the Phase I development program. With the advancement of mass spectroscopy, it is now possible for many NCE to carry out a significant part of the metabolic characterization using unlabeled NCE, as demonstrated by Xiao et al. (2016). The MAD study provides a better opportunity than the SAD for this work in that the drug will be at steady state.

A potential option in MAD studies is to take large plasma and urine samples at various times over a dosing interval at steady state and examine these for the presence of drug-related material via mass spectroscopy. Although this work may not completely eliminate the eventual need for a human radiolabeled metabolic study, it should allow such a study to be pushed back to late in the development process, when the success of the NCE is more certain.

It is also worth considering that current regulatory guidelines (FDA 2016; ICH-M3 (R2)) may require the quantification of major drug-related metabolites in both animal toxicology and human clinical studies. A knowledge of the human metabolism at a relatively early stage in the clinical development may therefore be critical to avoid having to repeat studies, purely because of a lack of exposure data.

Assessment of CYP3A4 Induction

Characterizing the potential of a NCE to induce CYP3A4 is an important part of the development process of any drug. In vitro studies can give a preliminary indication of the likelihood of induction potential but are not definitive. A commonly used approach has been to look at the change in the urinary ratio of 6β-hydroxy cortisol/cortisol before dosing and at steady state in the MAD study, although the results can be variable (Galteau and Shamsa 2003).

More recently, however, the use of plasma 4β-hydroxy cholesterol has proved a more robust technique (Diczfalusy et al. 2009) that has largely replaced the use of 6β-hydroxy cortisol/cortisol urinary ratio. For plasma 4β-hydroxy cholesterol, plasma samples are taken before dosing and at selected days during the study at pre-dose and a ratio of Day X/Day 0 then used to assess CYP3A4 status (Dutreix et al. 2013, 2014). For example, samples for 4β-hydroxy cholesterol could be taken at baseline, Day 3, Day 6, and Day 9s to 14 for a 2-week MAD study.

Example

To illustrate the type of data that can be obtained using a typical MAD, the results of an actual study are described below for NCE, ABC1234.

In this example, the pharmacokinetics of ABC1234 had already been studied in a SAD study in healthy male volunteers. Based on predictions of steady state, the available safety data and likely pharmacological levels, it had been decided to carry out a MAD study at four dose levels up to a maximum daily dose of 60 mg. Predictions indicated that steady state would be achieved before Day 14. The assay for the measurement of drug was adequately sensitive with a limit of quantification (LOQ) of 0.2 ng/mL. The study was carried out in a double-blinded manner in young healthy male volunteers, with the objective of characterizing the safety and pharmacokinetics of the compound after once-daily dosing for 14 days.

The compound was administered in hard gelatin capsules once daily for 14 days to groups of 12 subjects, 3 of which were matched placebos. A blood sampling regime, based on that from the SAD study, was used on Days 1 and 14. Pre-dose samples (C_{trough}) were taken from Days 2 to 14. After the last dose on Day 14, blood sampling was continued up to 72 h post last dose in order to characterize the terminal half-life at steady state. Previous human studies had indicated urinary excretion was $<5\%$ of the dose; thus, urine was not collected for the assessment of urinary pharmacokinetics. A pre-dose blood sample was collected from all subjects in order to genotype for metabolic polymorphisms, although the results were not considered in the current analysis.

The pharmacokinetic profiles of ABC1234 on Days 1 and 14 are shown in Figs. 1 and 2, with the approach to steady state given in Fig. 3. The derived pharmacokinetic parameters are shown in Tables 1 and 2, which included V_z/F, Cl_{ss}/F, and MRT. No evidence of a lag time in absorption (t_{lag}) had been observed in the SAD study, and the dosage form was identical; hence, it was not measured here. All blood samples were collected within $\pm 15\%$ of the theoretical sampling time specified in the protocol.

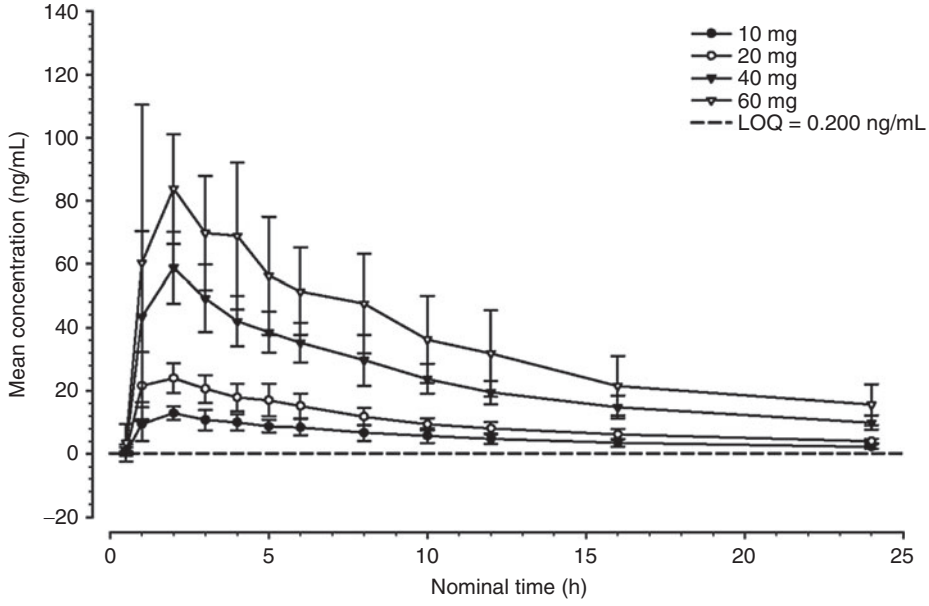

Fig. 1 Plasma concentrations (Mean ± SD) of ABC1234 after a single oral administration on Day 1

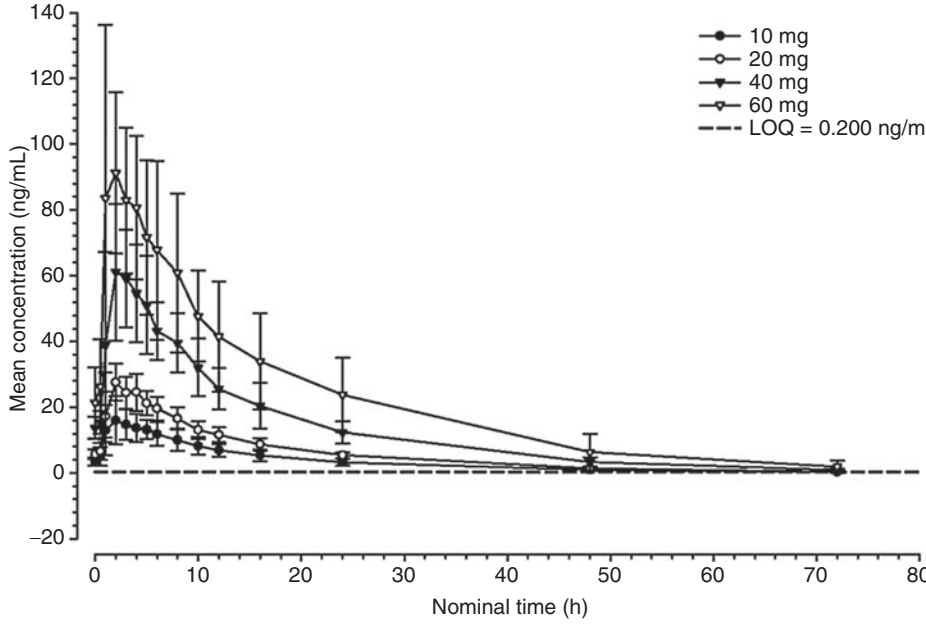

Fig. 2 Plasma concentrations (Mean ± SD) of ABC1234 at steady state on Day 14 after repeated daily oral administration for 14 days

ABC1234 appeared rapidly in plasma following oral administration of 10, 20, 40, and 60 mg of ABC1234 to healthy young male subjects. On Day 1, following a single oral administration, plasma concentrations were quantifiable up to the last sampling time (24 h post-dose) at all

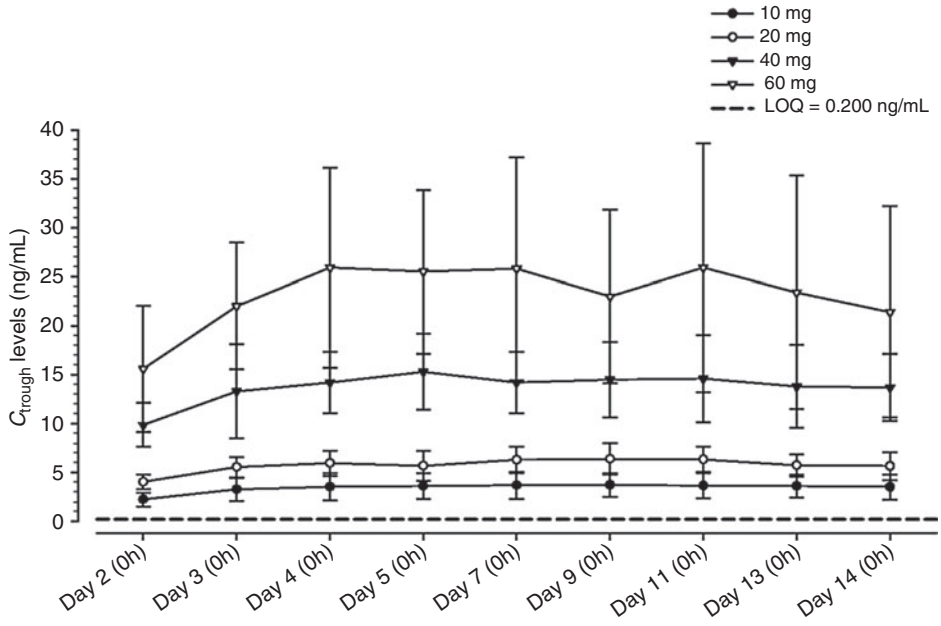

Fig. 3 C_{trough} plasma concentrations (Mean ± SD) of ABC1234 following daily administration for 14 days

Table 1 Pharmacokinetic parameters of ABC1234 after a single oral administration

PK parameter	10 mg	20 mg	40 mg	60 mg
C_{max} (ng/mL)	13.7 ± 2.76 (20) [13.5]	28.6 ± 5.09 (18) [28.1]	61.6 ± 13.6 (22) [60.2]	101 ± 26.3 (26) [97.7]
t_{max} (h)	2.00 (1.00, 3.00)	2.00 (1.00, 2.00)	2.00 (1.00, 2.00)	2.00 (1.00, 4.00)
AUC_{last} (ng h/mL)	129 ± 38.0 (29) [125]	234 ± 47.3 (20) [230]	558 ± 99.7 (18) [551]	840 ± 229 (27) [816]
$AUC_{0–24}$ (ng h/mL)	131 ± 38.3 (29) [126]	237 ± 47.7 (20) [232]	563 ± 100 (18) [556]	849 ± 233 (27) [824]

Tabulated values are Mean ± SD (CV%) [Geometric Mean] except for t_{max} where values are Median (Min, Max): Minor differences between AUC_{last} and $AUC_{0–24}$ reflect small variation in the actual sampling times around the 24 h point, but all were within ±15% of nominal

dose levels. On Day 14 following repeated daily administration, plasma concentrations were quantifiable up to the last sampling time (72 h post-dose) at the 20, 40, and 60 mg dose levels. ABC1234 was not quantifiable in any of the pre-dose Day 1 samples for subjects who received ABC1234 or in any of the samples analyzed from subjects who received placebo.

Mean C_{max} ranged from 13.7 ng/mL to 101 ng/mL on Day 1 and 18.1 ng/mL to 106 ng/mL on Day 14, over the dose range studied. The increase in C_{max} showed no major deviation from dose proportionality, although it should be noted that the lower 90% confidence interval for the β estimate of the log transformed power model was slightly greater than 1 on Day 1 (90% CI: 1.01,1.19). For a sixfold increase in dose, C_{max} increased by 7.20-fold on Day 1 (90% CI: 6.14, 8.45) and 6.23-fold on Day 14 (90%CI: 4.99, 7.79).

There was no accumulation of C_{max} over the dosing period (accumulation ratio pooled across doses was 1.08; 90% CI: 0.998, 1.16). Within-subject and total subject variability was low (19% and 26%, respectively).

Table 2 Pharmacokinetic parameters of ABC1234 at steady state on Day 14 after daily oral administration for 14 days

PK parameter	10 mg	20 mg	40 mg	60 mg
C_{max} (ng/mL)	18.1 ± 7.41 (41) [16.9]	27.8 ± 5.45 (20) [27.3]	66.3 ± 18.8 (28) [64.1]	106 ± 35.6 (34) [102]
t_{max} (h)	2.00 (1.02, 5.02)	2.00 (2.00, 3.00)	3.00 (1.00, 4.00)	2.00 (1.00, 6.00)
$t_{1/2}$ (h)	14.1 ± 2.33 (17) [13.9]	12.7 ± 1.09 (9) [12.6]	12.6 ± 0.978 (8) [12.6]	12.6 ± 2.13 (17) [12.4]
$AUC_{0–24}$ (ng h/mL)	190 ± 58.9 (31) [183]	313 ± 60.5 (19) [308]	723 ± 177 (25) [705]	1150 ± 402 (35) [1100]
V_z/F (L)	824 ± 211 (26) [802]	882 ± 151 (17) [871]	783 ± 186 (24) [763]	726 ± 213 (29) [698]
Cl_{ss}/F (L/h)	41.5 ± 11.7 (28) [39.9]	48.6 ± 9.28 (19) [47.8]	43.2 ± 10.3 (24) [42.1]	41.2 ± 13.6 (33) [39.0]
MRT (h)	17.4 ± 2.10 (12) [17.3]	16.7 ± 1.05 (6) [16.6]	16.4 ± 1.40 (9) [16.4]	17.3 ± 2.78 (16) [17.1]

Tabulated values are Mean ± SD (CV%) [Geometric Mean] except for t_{max} where values are Median (Min, Max)

Mean $AUC_{0–24}$ increased in a manner that was dose-proportional on both Days 1 and 14: For a sixfold increase in dose, $AUC_{0–24}$ increased by 6.80-fold on Day 1 (90% CI: 5.77, 8.02) and 6.19-fold on Day 14 (90% CI: 5.09, 7.52).

Consistent with the dose-proportional increase in C_{max} and $AUC_{0–24}$, V_z/F, Cl_{ss}/F, and MRT were independent of dose.

Over the dosing period, there was minimal accumulation of $AUC_{0–24}$ (accumulation ratio pooled across doses was 1.33; 90% CI: 1.29, 1.38). Within-subject and total subject variability was low, with values of 7.5% and 24%, respectively.

On repeated once-daily dosing, steady state was reached after the second or third dose at all dose levels (Fig. 3). Median time to steady state pooled across doses was 2.1 days (90th percentile 2.4 days).

Arithmetic mean $t_{1/2z}$ of ABC1234 on Day 14 following repeated daily administration was 12.6–14.1 h over the dose range studied. The difference in mean $t_{1/2z}$ between doses was not statistically significant ($p = 0.2333$), consistent with the dose-proportional nature of the pharmacokinetics.

In conclusion, ABC1234 appeared rapidly in plasma with a median t_{max} of 2–3 h and then declined with a terminal half-life of approximately 13 h. C_{max} and $AUC_{0–24}$ increased in a dose-proportional manner, reaching steady state by about Day 2. After repeated daily administration, there was minimal accumulation of $AUC_{0–24}$ (accumulation ratio: 1.33) over the dosing period. Within-subject and total subject variability for $AUC_{0–24}$ was low (7.5% and 24%, respectively).

References and Further Reading

Diczfalusy U, Kanebratt KP, Bredberg E, Andersson TB, Böttiger Y, Bertilsson L (2009) 4β-hydroxycholesterol as an endogenous marker for CYP3A4/5 activity. Stability and half-life of elimination after induction with rifampicin. Br J Clin Pharmacol 67(1):38–43

Dutreix C, Munarini F, Lorenzo S, Roesel J, Wang Y (2013) Investigation of CYP3A4-mediated drug-drug interactions on midostaurin in healthy volunteers. Cancer Chemother Pharmacol 72:1223–1234

Dutreix C, Lorenzo S, Wang Y (2014) Comparison of two endogenous biomarkers of CYP3A4 activity in a drug–drug interaction study between midostaurin and rifampicin. Eur J Clin Pharmacol 70(8):915–920

FDA (2016) Guidance for industry: safety testing of drug metabolites, revision 1

Galteau MM, Sharmsa F (2003) Urinary 6β-hydroxycortisol: a validated test for evaluating drug induction or drug inhibition mediated through CYP3A in humans and in animals. Eur J Clin Pharmacol 59(10):713–733

Gough K, Hutchinson M, Keene O, Byrom B, Ellis S, Lacey L, McKellar J (1995) Assessment of dose proportionality: report from the statisticians in the pharmaceutical industry/pharmacokinetics UK joint working party. Drug Inf J 29:1039–1048

ICH (2005) Gender considerations in the conduct of clinical trials
ICH M3 (R2) (2008) Nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals
ICH-E3 (1995) Structure and content of clinical study reports
ICH-E8 (1997) General considerations for clinical trials
Lui KA, Mager NA (2016) Women's involvement in clinical trials: historical perspective and future implications. Pharm Pract (Granada) 14(1):708. 1–9
Rowland M, Tozer TN (2011) Clinical pharmacokinetics and pharmacodynamics: concept and applications, 4th edn. Lippincott Williams & Wilkins, Philadelphia
Xiao W, Shen G, Zhuang X, Ran X, Zhu M, Li H (2016) Characterization of human metabolism and disposition of levo-tetrahydropalmatine: qualitative and quantitative determination of oxidative and conjugated metabolites. J Pharm Biomed Anal 128:371–381