Population pharmacokinetics of APOMINE™: A meta-analysis in cancer patients and healthy males

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Aims

1) To characterize the population pharmacokinetics of apomine in healthy males and in male and female patients with solid tumours and 2) to understand more fully the influence of induction and between- and within-subject variability on exposure to drug using Monte Carlo simulation.

Methods

Apomine was administered once- or twice-daily with or without food in single and multiple oral doses of 30–2100 mg to healthy males ($n = 19$) and patients with solid tumours ($n = 19$). The data were divided into model development and validation sets. Models were developed using standard population methods. These were the identification of an appropriate base model, calculation of the empirical Bayes estimates of the primary pharmacokinetic parameters, covariate screening, forward step-wise addition of covariates using the likelihood ratio test as a model selection criteria, and backwards elimination to obtain the final model. To study the influence of data from individual subjects, the model development dataset was subjected to the delete-1 jack-knife and the final model was fitted to each jack-knifed dataset. Principal components analysis of the jack-knifed matrix of model parameters identified two influential subjects who were removed from the dataset, and the final model contained data from the remaining subjects. Model validation was examined using goodness of fit statistics and relative error measures using independent datasets from cancer patients. The model provided a reasonable approximation to the pharmacokinetic measurements in the validation datasets. Computer simulations were undertaken to understand further the pharmacokinetics of apomine in otherwise healthy females, a population not yet studied.

Results

Apomine pharmacokinetics were complex and consistent with a two-compartment model with a lag-time. Apparent oral clearance at baseline and apparent volume of distribution at steady-state were larger in healthy males than in cancer patients (41 ml h⁻¹ and 14.1 l vs 10 ml h⁻¹ and 8.9 l, respectively, for a 75 kg person). Clearance was time-variant showing a maximal increase with full induction of 320 ml h⁻¹, independent of patient type. The time to reach 50% maximal induction was about 2 days. The fraction of drug absorbed was relatively constant at doses less than 100–200 mg once daily but decreased at higher doses. Food also decreased relative bioavailability by 36%. Patient characteristics had no effect on apomine pharmacokinetics except for weight, which was proportional to the volume of the central compartment. Between-subject variability (68% for clearance, 30% for central volume, and 141% for peripheral volume) was moderate to large and independent of patient type. Inter-occasion variability was small (18% for both clearance and central volume). Residual variability was modelled with an additive and proportional error model. Cancer

patients had slightly higher plasma concentrations than healthy males but this difference was probably not clinically significant. Steady-state was reached in about 3–4 days after once-daily drug administration. The half-life of apomine after three weeks of once-daily dosing was 41 h in cancer patients and 32 h in healthy males.

Conclusions

A population model for apomine has been developed that characterizes its pharmacokinetics in cancer patients and healthy subjects under a variety of conditions.

Introduction

Apomine™ (SR-45023 A), a bisphosphonate ester with multiple pharmacological properties, was originally studied as an antihypercholesterolaemic agent. One of its unique mechanisms of action is to decrease cholesterol synthesis by accelerating HMG-CoA reductase degradation [1]. Another action is the activation of the farnesoid nuclear receptor (FXR), which has been implicated in inducing apoptosis [2]. *In vitro* studies indicated that apomine inhibited cell growth in a variety of cell lines with an IC_{50} ranging from 2.8 to 11 $\mu\text{g ml}^{-1}$ (unpublished data). These results were later confirmed *in vivo* using SW-620 and HT-29 xenograft models (unpublished data). Apomine has been studied in cancer patients as a cytostatic agent [3]. In addition, apomine has been shown to increase bone volume, mineral content, and bone specific alkaline phosphatase (a marker of bone formation) in mice [4]. Furthermore, an increase in bone-specific alkaline phosphatase has been observed in response to apomine treatment in some cancer patients. Based on these *in vivo* results and additional *in vitro* studies, the effect of apomine on bone remodelling is currently being studied in women with osteoporosis or low bone mass.

The pharmacokinetics of apomine have been studied after different single and multiple doses, with food and in the fasting state, in cancer patients and healthy subjects. Preliminary pharmacokinetic data have been reported in seven patients with solid tumours [3]. The purpose of the present analysis was to characterize the pharmacokinetics of apomine in cancer patients and healthy male subjects and to identify those patient characteristics that may influence its pharmacokinetics. Secondary aims were to predict the pharmacokinetics of apomine in healthy females using Monte Carlo simulation and to develop a rationale dosing regimen for future studies.

Methods

Clinical studies

Data from six Phase I and Phase II clinical studies were available at the time of model development. Data

from another study (Study 7) then became available. Table 1 presents a summary of the studies and their experimental designs. The range of doses used varied from 30 mg to 2100 mg. The drug was given either once or twice daily with or without food. All studies involved intensive pharmacokinetic sampling and were considered data rich. Table 2 presents a demographic summary of the patients in each study. In Studies 1 and 2, healthy subjects were hospitalized in a Phase I clinical unit. Doses were administered by nursing staff and the timing of samples was measured exactly. All other studies were performed on an out-patient basis. In Study 5, the first 4 weekly doses were administered by hospital staff with all subsequent doses administered by the patient. In all other studies, the drug was administered by the patient, except on pharmacokinetic sampling days, when the drug was either administered or ingestion witnessed by the nursing staff. The exact time of blood sample collection was not recorded in all studies. Cancer patients received concomitant medications for supportive care with the exception of chemotherapy, immunotherapy, hormonal cancer therapy, radiation therapy, or other experimental medications. All patients gave written informed consent prior to enrolment and all studies were done in accordance with Declaration of Helsinki and Good Clinical Practices. The studies were approved by the following local IRBs/ethics committees: Tayside Committee on Medical Ethics, Dundee, Scotland (Studies 1 and 2); Arizona Cancer Center, Tucson, AZ, USA (Studies 3 and 4); Cancer Therapy and Research Center, San Antonio, TX, USA (Studies 5 and 6); and Charing Cross Hospital, London, UK (Study 7).

Analysis of apomine

Apomine concentrations were assayed using a validated gas chromatographic assay with nitrogen-phosphorous detection (GC/NPD) [3]. The assay has a linear range of 0.01–50 $\mu\text{g ml}^{-1}$ and a coefficient of variation of less than 15%. The lower limit of quantification of the assay was 0.01 $\mu\text{g ml}^{-1}$.

Table 1
Study characteristics

Study	Food	Design	Sex	Sample collection times relative to first dose	Cancer	<i>n</i>	Use
Study 1	Fasting	Single dose	Males	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 30, 48, 72, 96, 168, 336, 672, 1344 h	No	15	Development
Study 2	Fasting	Multiple dose qd	Males	0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 52, 72, 76, 96, 100, 120, 124, 144, 148, 168, 172, 196, 216, 220, 240, 244, 264, 268, 288, 292, 312, 316, 336, 336.5, 337, 338, 339, 340, 342, 344, 348, 360, 364, 408, 456, 504, 672, 840 h	No	4	Development
Study 3	Food	Multiple dose bd	Males and females	Day 8: 0 h Day 14: 0, 0.5, 1, 2, 4, 6, 8, 12 h Day 15: 0 h	Yes	14	Validation
Study 4	Food	Multiple dose bd	Males and females	Day 8: 0 h Day 14: 0, 0.5, 1, 2, 4, 6, 8, 12 h Day 15: 0 h	Yes	14	Validation
Study 5	Fasting	Once weekly	Males and females	Day 1: 0, 0.25, 1, 2, 3, 4, 6, 8, 12 h Day 2, 3, 4, 5, 8, 15, 22, 29: 0 h	Yes	11	Development
Study 6	Food	Multiple dose bd	Males and females	Day 1: 0, 0.25, 1, 2, 3, 4, 6, 8, 12 Day 2, 3, 4, 5, 8: 0 h	Yes	8	Development
Study 7	Food	Multiple dose bd	Males and females	Day 1: 0, 0.25, 1, 2, 3, 4, 6, 8, 12 Day 2, 3, 4, 5, 8, 15: 0 h	Yes	15	Validation

Total number of subjects in model building set: 38, total number of subjects in model validation set: 43.

Table 2
Demographic data

Study	Age (years) ^a	Weight (kg) ^a	Sex ^b
Study 1	25.5 (18–42)	71.9 (55–94)	15 M
Study 2	27.3 (22–31)	72.7 (69–81)	4 M
Study 3	57.9 (37–74)	Missing	6 M/8 F
Study 4	58.1 (32–79)	Missing	5 M/9 F
Study 5	52.8 (23–76)	80.3 (55–115)	4 M/7F
Study 6	60.1 (46–78)	75.6 (58–96)	5 M/3 F
Study 7	58.0 (31–76)	68.6 (51–114)	4 M/11 F

^aData are reported as mean (minimum–maximum). ^bM, males, F, females.

Model development

Models were developed using standard population pharmacokinetic methodology. First, a structural base model without covariates was identified. The empirical Bayes estimates were calculated and used to screen for important covariates, which were added until a final covariate

model was identified. Influential observations were isolated and removed from the covariate model through a delete-1 jack-knife analysis of the final model. The covariate model without the influential observations was deemed the final model.

All model development was done using NONMEM (version 5, Build 1) using first-order conditional estimation with interaction. The data from studies 1, 2, 5 and 6 were used in model development. Data from studies 3 and 4 were used in model validation since these datasets were missing body weight information for all subjects. After model development was complete, another dataset (study 7) became available, which was also used for model validation.

Base model development

The base model was developed from the concentration-time data, the physicochemical properties of the drug and from preclinical observations. In study 1 (single dose) dose-normalized AUC and $C_{\max}$ decreased with increasing dose, reaching a minimum at 150 mg, which suggests that saturable absorption was occurring. This is consistent with the low solubility of apomine

(<0.1 µg ml⁻¹ in water), because it was expected that drug absorption would be dissolution rate limited and that increasing the dose would eventually saturate gastrointestinal fluids.

Because apomine is poorly water soluble and highly lipophilic (mlogP = 6.8), it was also expected that food would affect relative bioavailability (F_1). Hence, three models of F_1 were examined:

$$F_1 = \left[1 - \frac{\text{Dose}}{D_{50} + \text{Dose}} \right] (1 + \text{Food} * \theta_{\text{food}}) \quad (1)$$

$$F_1 = \left[1 - \frac{(\text{Dose})^n}{(D_{50})^n + (\text{Dose})^n} \right] (1 + \text{Food} * \theta_{\text{food}}) \quad (2)$$

$$F_1 = [1 - \exp(-D_{50} * \text{Dose})] (1 + \text{Food} * \theta_{\text{food}}) \quad (3)$$

where D_{50} is the dose that produces a 50% decrease in relative bioavailability, n is the shape parameter, Food is a binary dummy variable indicating whether the dose was taken without (equal to 0) or with food (equal to 1), and θ_{food} is the estimable parameter associated with a food effect.

Given the solubility and lipophilicity properties of apomine, a simple first-order absorption model might not be applicable. Thus, a number of different models were tested, including:

- First-order absorption;
- First-order absorption with lag-time;
- Time-dependent first-order absorption using a change-point model [5] with and without lag-time;
- Time-dependent first-order absorption using a Bateman function [5] with and without lag-time;
- Zero-order absorption;
- Simultaneous first- (with and without lag-time) and zero-order absorption;
- First-order absorption (with and without lag-time) treated as a mixture model.

In the case where the distribution of absorption rate constants and lag-time were treated as a mixture model, and to ensure that the proportion of subjects assigned to one group did not exceed the boundary conditions [0, 1], the mixing proportion was defined as:

$$P(1) = \frac{1}{1 + \exp(P1)} \quad (4)$$

where $P(1)$ is the proportion of subjects assigned to group 1 and $P1$ is an estimable parameter on the interval [-5, 5].

The half-life of apomine after single dose administration was estimated to be 275–350 h using mean concentration-time profiles. However, after repeated dose

administration (study 2), steady-state was achieved within a few days of dosing, which cannot be explained by stationary, linear pharmacokinetics. Following oral administration for 28 days in dogs and rats, apomine was shown to induce various isoforms of cytochrome P450 and testosterone 6β- and 2β-hydroxylases (unpublished data). Furthermore, increased liver weight, liver enzyme activity and hepatocellular and thyroid follicular cell hypertrophy, all markers of hepatic induction, were observed. Although induction *in vivo* has not been confirmed, an induction model was tested allowing apparent oral clearance to vary over time to determine whether induction improved the goodness of fit. Auto-induction has been modelled using both mechanistic [6], semimechanistic [7], and empirical models [8]. Attempts to model auto-induction using a mechanistic model failed, and thus an empirical approach was used. Apparent oral clearance was modelled using a sigmoid E_{max} model with baseline, using time relative to the first exposure to apomine as the independent variable. It was assumed that only subjects who received multiple doses of apomine would show induction. Hence, apparent oral clearance was treated as time-invariant for subjects in the single dose study.

To allow for differences in pharmacokinetics between healthy subjects and patients, typical values for all distribution- and elimination-related parameters (e.g. clearance and volume) were allowed to vary between groups. It was also assumed that the variability within subjects in the healthy group and cancer groups could also be different. However, if the ratio of the variance components between healthy subjects and cancer patients was less than 4, a common variance was assumed between groups. The value of 4 was chosen because this is a common heuristic used to assess equality of variances based on critical values for Hartley's F-max test.

Some patients showed dual peaks in the concentration-time profile about 8–12 h after a single dose of apomine, which is consistent with enterohepatic recycling. Apomine has a molecular weight of 563 Da, and drugs with values greater than 500 Da tend to be cleared exclusively by metabolism and biliary secretion [8]. Many models have been developed to explain enterohepatic recycling, most using some form of catenary compartment model between the elimination compartment and absorption compartment [10–12]. No suitable model could be developed when applied to the present data, and it was decided for the sake of expediency that this aspect of the model would be not taken into account.

A wide range of concomitant medications were taken by the cancer patients studied whereas co-administered drugs were not allowed in healthy subjects. Most of the

concomitant medications in the cancer patients were antibiotics, analgesics, antiemetics, over the counter medications, and few were CYP3A substrates. No patients were taking CYP3A inhibitors and only the occasional use of an antacid was found. Given the relatively small number of patients in the database and the difficulty of detecting a drug–drug interaction of small magnitude, it was decided to ignore the potential effect of concomitant therapy. Future Phase III studies with greater numbers of subjects and where the statistical power to detect such interactions is larger, will allow the effects of other drugs on the pharmacokinetics of apomine to be examined.

It is possible that vomiting could affect the pharmacokinetics of apomine by altering drug absorption. Although no healthy subjects experienced vomiting during the study, some cancer patients did. A review of the database showed 10 instances of vomiting (7 Grade 1, 2 Grade 2, 1 Grade 3) in six individuals during Cycle 1. Unfortunately, the exact time of vomiting in relation to dosing was not recorded. Since the number of vomiting episodes was small relative to samples taken and doses given and its severity was mostly low grade, the effect of vomiting was ignored during model development.

Lastly, the model needed to accommodate all sources of variability, namely between-subject variability (BSV), interoccasion variability (IOV), and residual variability. BSV and IOV were modelled as a log-normal distribution, whereas residual variability was modelled using an additive plus proportional error model. Different residual variance models were tested periodically during model development, but in all cases failed to improve the goodness of fit of the model. When a new parameter was added to the model, it was treated as a random effect. If the associated variance component had a coefficient of variation (CV) less than 3% (corresponding to an ω^2 of 0.001), the associated variance component was removed from the model.

One-, two-, and three-compartment models were examined. Typical pharmacokinetic parameters and variance components for cancer patients and healthy subjects were allowed to vary. IOV was not included in the model at this point. When an appropriate structural compartment model was found, a relative bioavailability component to the model was added [equation (1.1) to (1.3)]. When an adequate relative bioavailability model was identified, an induction model was added. Once a suitable induction model was found, IOV was added to all parameters with an occasion being defined as the day of sampling. This represented the best base model. To ensure that a component was not mis-specified, alternative relative bioavailability and induction models were

varied in combination. If an alternative model was superior to the best base model, then the alternative model was made the best base model. Random effects that were less than 3% CV were removed from the model if this had not already been done through rounding errors in their estimation.

Covariate screening and covariate model development

Once the base model was identified, the empirical Bayesian estimates for the primary pharmacokinetic parameters were estimated. Using graphical assessments, screening was performed to identify those covariates that might influence the pharmacokinetic parameters. The covariates available were age, sex, weight, race, dose, presence or absence of food within 30 min prior to dose administration, and cancer patient or healthy subject status. Those covariates identified by graphical analysis as having a potential influence on a particular parameter were added to the model sequentially using stepwise regression techniques. The order of covariate addition to the model was based on the perceived influence on goodness of fit. For example, dose was deemed to be the most important covariate through its effect on $F1$ and thus it was included first, followed by patient or healthy subject status, presence or absence of food, weight, sex, age, and race. Continuous covariates were standardized to their mean to improve the stability of the optimization process. Nested models were compared using a critical value of 0.05 with the likelihood ratio test. Covariates that significantly reduced the log-likelihood were retained. Once the final model was identified, backwards regression using a 0.01 significance level was used to confirm the significance of a covariate.

Identification of influential observations and subjects

Influential observations were identified through examination of the weighted residuals. Weighted residuals having an absolute value greater than five were removed from the model. Influential subjects were identified by generating jack-knifed datasets where a unique subject was removed from each one. The final model was applied to each jack-knifed dataset. Principal components analysis (PCA) was performed on the jackknifed matrix of model parameters [13], more specifically on their estimable log-transformed absolute values (to ensure positivity), typical values and variance components, separately. Using the criteria of Karlis *et al.* [14] only those eigenvalues greater than

$$\hat{\lambda} > 1 + 2\sqrt{\frac{p-1}{n-1}} \quad (1.5)$$

were retained, where p is the number of estimable model parameters and n is the number of subjects the covariance matrix was based on. Plots of the retained principal components were used to identify influential subjects who were then removed from the original, un-jack-knifed dataset. The final covariate model was then applied to the dataset without influential subjects and the resulting parameter estimates were considered final.

Model validation

Model validation was done using datasets from studies 3, 4, and 7. Study 7, which should have been a candidate for the model development set, only became available after modelling was completed. Datasets from studies 3 and 4 were used in model validation because body weight data were not collected in these studies and, thus, could not be used as a covariate (unless it was imputed) in model development. For the purposes of validation, the body weight of all subjects in these two studies was made equal to the population mean weight for males and females observed in the model development set. Model validation was done by examining goodness of fit plots and comparison of the mean and variance of the 10% trimmed relative error (observed vs predicted concentrations) compared with the metrics obtained using the model validation dataset.

Simulations

To understand more fully the influence of induction and between- and within-subject variability on exposure, Monte Carlo simulation was also used to estimate apomine half-life, AUC(0,24 h), and $C_{\max}$ during 21 days of therapy. Concentration-time data were simulated using once a day dosing of 50 mg apomine in 50 fasting healthy female subjects. Weight was treated as a normally distributed random variable with mean and variance equal to the population mean and variance for females observed in the model development set. Half-lives were determined using standard formulae for estimating the macroconstants of a two-compartment oral open model [15]. On each day of dosing, AUC(0,24 h) was calculated using the linear trapezoidal rule and $C_{\max}$ was determined from direct observation of the data.

Results

The model development set consisted of data from 38 subjects consisting of a total of 801 plasma concentrations. Apomine pharmacokinetics were best described by a two-compartment model. Of the absorption models tested, a simple first-order model fitted the data best, where the absorption rate constant and lag-time were

treated as a mixture model. Group 1 had an estimable lag-time and absorption rate constant whereas group 2 had no lag-time (i.e. lag-time = 0), but did have an estimable absorption rate constant. Relative bioavailability (F_1) was best described using an $E_{\max}$ model that was influenced by food [equation (1.1)]. Clearance and central volume of distribution were best described by allowing typical values to differ between cancer patients and healthy subjects, with both groups having common between-subject variability. No difference was observed between groups for intercompartmental clearance and peripheral volume of distribution. Thus typical values and their associated variance components were treated as the same for both groups. Clearance was time-dependent and best characterized using a sigmoid $E_{\max}$ model with baseline. Although the time to reach 50% maximal induction (t_{50}) was treated as a random effect, the maximal increase in clearance with full induction ($CL_{\max}$) was treated as a fixed effect. No covariate effects were associated with t_{50} and the induction model was the same between cancer patients and healthy volunteers. Examination of the histogram for peripheral volume of distribution showed that four subjects had values much higher than all other subjects. These four subjects were in study 2 (healthy, male multiple-dose study). No definitive explanation for these results could be found, although the difference may be due to variations in sampling schemes. However, to control for the bi-modality of the distribution, peripheral volume of distribution (V_3) was allowed to vary from the typical value for this group of subjects (θ_{V_3}) using the equation

$$V_3 = \theta_{V_3} \theta_{\text{Study2}} * \exp(\eta_{V_3}) \quad (1.6)$$

where θ_{Study2} is the multiplier for the group. Between-subject variability was modelled log-normally and was estimated for clearance, central compartment volume of distribution, and peripheral compartment volume of distribution, but could not be determined for inter-compartmental clearance. Inter-occasion variability was estimated for clearance and central volume of distribution. Residual variability was modelled using an additive and proportional error model.

The first three principal components for the jack-knifed log-transformed model parameters and variance components accounted for 66% and 69% of the total variance, respectively. Principal components analysis revealed that two subjects (1.18 and 5.11) had a consistent effect on the estimates of the typical values and the variance components (Figure 1). These subjects were removed from the dataset and the final model parameter estimates are shown in Table 3. Goodness-of-fit plots

Figure 1

Scatter plots from principal components analysis of the final jack-knifed covariate model for estimable parameters (upper panel) and variance components (lower panel). Suspect values are indicated by identifiers (study number. subject number, e.g. 1.18 refers to Study 1, Subject 18)

revealed good concordance between observed and predicted values (Figure 2). Residual plots failed to detect any trends or influential observations (Figure 3). None of the observations had a weighted residual greater than ± 5 , and so no observations were removed from the dataset as suspected outliers. Representative plots of observed concentrations and model predicted concentrations are shown in Figure 4. The condition number (ratio of largest to smallest eigenvalue) of the final model was 2880, indicating that the model was stable and not ill-conditioned. The distribution of the 10% trimmed relative errors was approximately normal with a mean of 1.0% and a range of -24% to 31%.

Goodness-of-fit plots for the validation datasets also appeared to be adequate with slightly more variability than the model development dataset (Figures 5 and 6). The 10% trimmed relative errors for studies 3 and 4, based on 251 observations from 28 subjects, was approximately uniform in distribution with a mean of -0.5% and a range of -13 to +13%. The 10% trimmed relative errors from study 7, based on 360 observations from 15 subjects, was approximately normal in distribution with a mean of -0.1% and a range of -25 to +27%. Overall, the model appeared to characterize adequately

the pharmacokinetics of apomine in a variety of different dosing conditions and populations.

Healthy subjects had higher clearance and central volume of distribution compared with cancer patients with no difference in intercompartmental clearance or peripheral volume of distribution. Both healthy subjects and cancer patients showed induction of metabolism with 50% of maximal effect occurring within about 2 days after repeated administration of apomine. Because of induction, clearance and, hence, the λ_1 - and λ_z -half-lives changed over time. The λ_1 -half-life was reasonably robust to the change in clearance and remained constant for most patients over the induction period (Figure 7). The λ_z -half-life was far more sensitive to changes in clearance. After repeated administration, due to the large shape parameter associated with the induction model, the λ_z -half-life decreased together with its between-subject variability (Figure 7). Also, the difference in clearance and central volume of distribution between cancer patients and healthy subjects resulted in different half-lives in these groups. The least-squares mean λ_1 -half-life at steady-state in cancer patients and in healthy volunteers was 5.5 h and 4.5 h, respectively and the least-squares mean λ_z -half-life was 41 h and 32 h, respectively.

Table 3

Pharmacokinetic parameters based on the final covariate model

Submodel	Parameter	Value	SE	BSV (%)*
Healthy males	Clearance or CL_0 (ml h ⁻¹)	40.7	8.07	68
	Typical central compartment volume (l) or TVV2	12.3	1.86	30
Cancer patients	Clearance or CL_0 (ml h ⁻¹)	10.2	3.87	68
	Typical central compartment volume (l) or TVV2	7.11	2.13	30
Induction model	$CL_{\max}$ (ml h ⁻¹)	320	85.3	**
$CL = CL_0 + CL_{\max} \times \text{Time}^n / (t_{50}^n + \text{Time}^n)$	t_{50} (h)	46.4	17.6	88
	Shape parameter (n)	6.40	1.76	**
Bioavailability (F) model	$F1_{\max}$	1	fixed	**
$F = [1 - F1_{\max} \times \text{Dose} / (\text{Dose} + D_{50})] \times (1 + \theta_{\text{food}} \times \text{Food})$	D_{50}	128	39.4	**
	Food effect on $F1$ (θ_{food})	-0.360	0.119	
Absorption model	Absorption rate constant (Group 1) (h ⁻¹)	1.77	0.431	145
	Lag time (Group 1) (h)	0.821	0.0386	**
	Absorption rate constant (Group 2) (h ⁻¹)	0.361	0.0362	145
	Lag time (Group 2) (h)	0	fixed	**
Distribution parameters common to both groups	$P1^{***}$ [See equation (1.4)]	-3.47	1.15	
	Intercompartmental clearance (ml h ⁻¹)	198	40.9	**
	Power term for weight on central compartment volume	1.00	Fixed	
	Peripheral compartment volume (l)	1.83	0.679	141
Inter-occasion variability	Peripheral compartment multiplier for study 2	23.5	7.42	
	Clearance			18
Residual variance model	Central compartment volume			18
	Proportional error			11
Observed = Predicted*exp(e1) + e2	Additive error	0.168		

* Denotes common variability for both cancer patients and healthy males; ** Denotes variance too small to estimate; *** Denotes the mixing fraction was 97% into group 1 and 3% into group 2. Central compartment volume was modelled as $V2 = TVV2 \left(\frac{\text{weight}}{75 \text{ kg}} \right)^{1.0}$. CL apparent oral clearance; CL_0 apparent oral clearance in the absence of induction.

Figure 8 shows the simulated 24 h AUC and $C_{\max}$ vs time as metrics of exposure to apomine in fasting female subjects dosed once daily with 50 mg of the drug. Both AUC and $C_{\max}$ increased for about the first week of administration, and then decreased and reached steady-state after about 2 weeks of administration. By about day 14, exposure was approximately twice that seen with the first dose. Thus, despite such a long initial half-life, there was little predicted accumulation of the drug after about 2 weeks' treatment. Between-subject variability also seemed to become larger as time progressed, such that by the third week of treatment there was approximately 10-fold variability in $C_{\max}$ and 30-fold variability in AUC.

Apomine can be characterized as having moderate between-subject variability and small interoccasion variability. Between-subject variability for clearance and central volume of distribution was 68% and 30%, respectively, regardless of patient type, whereas interoccasion variability was only 18% for both clearance and

central volume of distribution. A difference was also observed in the rate of induction of metabolism, with the variability in t_{50} across subjects being 88%. Accordingly, the time for clearance to stabilize becomes longer as t_{50} increases. Based on the simulated data, the time to reach a stabilized clearance was approximately 1 week for all patients.

Discussion

The pharmacokinetics of apomine are complex. The fraction of drug absorbed was relatively constant at doses of less than 100–200 mg given once daily but decreased as the dose was increased. A likely reason for this deviation from dose proportionality is the low solubility of apomine (<0.1 µg ml⁻¹) and saturation of gastrointestinal fluids at high doses. Food also decreased plasma concentrations of apomine, possibly due to non-specific binding of the drug to food constituents in the gastrointestinal tract.

Figure 2

Observed vs individual model predicted concentrations (top) and mean predicted model concentrations (bottom) for the model development dataset. The dashed line is the line of unity

Figure 3

Residual plots of weighted residuals vs square root time (top) and weighted residuals vs individual predicted concentrations (bottom) for the model development dataset

Clearance and central volume of distribution were larger in healthy subjects than cancer patients. One possible reason for this observation is that cancer patients will have taken many more concomitant medications than the healthy subjects and these may have affected the pharmacokinetics of apomine. A second reason may be that the cancer patients were generally older than the healthy subjects. It is well known that clearance tends to decrease with age for most drugs. However, age was tested as a covariate in the model and found not to improve the goodness of fit. The likelihood of age-related differences in clearance, although small, cannot be ruled out. A third possible reason is a difference in protein binding between cancer patients and healthy subjects. More than 99% of apomine binds to plasma proteins and the drug has an especially high affinity for human α_1 -acid glycoprotein (unpublished data), which

is restrictive and saturable. α_1 -acid glycoprotein tends to be found in higher concentrations in cancer patients than in healthy subjects [16]. Hence, cancer patients should have less free drug in their plasma. The effect of this increase in binding will be two-fold. First, apomine clearance should be smaller in cancer patients, since only free drug is available for elimination. Second, since volume of distribution of the central compartment is a function of the unbound fraction in plasma, the volume of distribution would be expected to decrease in cancer patients.

No difference in intercompartmental clearance and peripheral volume of distribution was observed between groups. Intercompartmental clearance is more a function of tissue perfusion whereas peripheral volume of distribution is determined by unbound fraction of drug in the tissues. There is no evidence that these two variables

Figure 4

Scatter plots of observed concentrations (●) and model predicted concentrations (—) in four representative subjects from different studies based on the final covariate model

are different between the two populations and, hence, no difference between groups in clearance and volume would be expected

The only important patient-specific covariate identified in the analysis was body weight, which was proportional to central volume (V_2), as described by the following equation:

$$V_2 = \theta_{V_2} \left(\frac{\text{Weight in kg}}{75 \text{ kg}} \right)^{1.00} * \exp(\eta_{BSV,V_2} + \eta_{IOV,V_2}) \quad (1.6)$$

where θ_{V_2} is the intercept common to both groups, η_{BSV,V_2} represents between-subject variability, and η_{IOV,V_2} interoccasion variability. The power term was fixed at unity since its initial estimation was very close to 1.00 and theoretical considerations indicate that volume increases proportionally with log weight. Apomine is highly lipophilic and would be expected to have a volume of distribution proportional to body fat. Since body

weight is related to the amount of body fat, compartment volumes would naturally be expected to be proportional to body weight or other surrogates for body fat, such as lean body mass or possibly even body surface area.

Peripheral volume appeared to be bimodally distributed with a small group of subjects (all in study 2) having values many times higher than the rest of the population. To account for this difference a dichotomous covariate was created where subjects in the other studies were given one typical value, and subjects in study 2 were given a different one. This model significantly improved the fit and showed that subjects in study 2 had a peripheral volume 23-times higher than the other patients. Even though this difference appeared large, simulations (data not shown) indicated that the difference in concentration-time profiles was minimal.

An induction model was invoked to account for the rapid attainment of steady-state (within a matter of days) and to account for preclinical results. Many induction models are available, most requiring differential equations, which would have increased computer times to

Figure 5

Goodness of fit plot for the validation datasets from study 3 and study 4 (left) and study 7 (right). The dashed line is the line of unity

Figure 6

Residual plots of weighted residuals vs square root time for the validation datasets from study 3 and study 4 (left) and study 7 (right)

Figure 7

Line plot of apomine λ_1 -half-life (top) and λ_2 -half-life (bottom) as a function of day of drug administration

Figure 8

Line plot of apomine AUC(0,24 h) (top) and $C_{\max}$ (bottom) as a function of day of drug administration

unrealistic lengths. However, none of these models was successfully implemented and an empirical model was used instead. The time to reach 50% maximal induction was about 2 days. Many other reports have indicated that enzyme induction is a relatively rapid process. Ohnhaus & Park [17] reported that rifampicin, a classic enzyme inducer, causes 50% maximal induction in about 3 days and CYP3A was 50% maximally induced by ifosfamide within 13 h [7]. The time course of induction by apomine is consistent with that of other enzyme inducers. However, no evidence of dose-dependency of the maximal rate of induction was observed, as had been seen with some inducers [18]. A dose-response in the maximal induction by apomine is likely to occur but could not be detected using the present study designs.

In this population analysis, we modeled apomine clearance as an induction process because of the observed enzyme induction in animals and because of the large doses given to humans. An alternative to an

induction model would be one that included saturable, nonreversible protein binding as the rate limiting factor in the clearance of apomine. Apomine is very tightly bound to human α_1 -acid glycoprotein which would restrict the removal of apomine from the body. It should be noted that apomine does not bind to rat, dog or monkey α_1 -acid glycoprotein which explains the very different pharmacokinetic profile observed in humans (unpublished data). The restricted binding in humans compared to animals would potentially limit the level of exposure to the liver for metabolism and thus apomine may also have a very different metabolic profile in man compared with animals. However, there is a limited pool of α_1 -acid glycoprotein available for binding so that with repeated dosing and apomine accumulation, saturation of α_1 -acid glycoprotein would eventually occur. The result would be that more free drug would be available for elimination which would manifest in an increase in clearance. A mathematical function to model

such a process would be equivalent to the induction model presented in Table 3, but now $CL_{\max}$ would represent the maximal increase in clearance when all α_1 -acid glycoprotein available for binding was saturated and t_{50} would be the time for 50% of available α_1 -acid glycoprotein to be saturated (assuming α_1 -acid glycoprotein concentrations were at steady-state). As there was evidence of induction in animals, the observed change in clearance over time was labeled an induction process, however, without further data on the metabolism of apomine in man, it would be difficult to distinguish between the induction model and saturable, protein binding model kinetically.

One limitation of the present pharmacokinetic model was failure to control for the double concentration-time peaks observed after single dose administration, which probably reflects enterohepatic recycling. The timing of the second peak is consistent with gall bladder emptying after meals [9]. A number of models have been proposed for recycling but they could not be successfully implemented in the present analysis. Hence the decision was made to ignore the double peaks. The effect of not taking account of recycling had little effect on the predictive capabilities of the resulting model. However, the estimates of central volume and clearance are probably biased to a small degree.

Apomine exhibited moderate between-subject variability for clearance (68%) and central volume (30%). More importantly, interoccasion variability was considerably smaller (<20%), indicating that large fluctuations in concentration from one dose to the next were not observed within subjects. The first-order absorption rate constant showed larger between-subject variability, most likely because of model misspecification of the absorption phase. It seems unlikely that oral absorption is a first-order process to all the doses studied. Absorption probably changes zero-order at higher doses. Although, many different absorption models were tried, none of them fitted the data better than a first-order model. The current model was a reasonable approximation over a range of doses given by different regimens to different populations.

The identification of influential subjects in a population analysis is a difficult task and one that has received scant attention in the literature. One method to assess the effect an individual subject has on a model is to remove the data from that subject and re-fit the model to the remaining data. A stable model is one that gives little change in the parameter estimates when a subject is removed. However, the chances of a spurious change are high, when a large number of different parameters are being estimated. Hence, the problem

becomes one of data reduction, and the solution is to decrease the matrix of jack-knifed parameters into fewer components.

Principal components analysis converts the number of dimensions in the data into a few components that contain as much of the variance in the original data as possible. Each component is independent of the other components. The generation of the principal components is a function of the mean and variance of the original matrix. Many pharmacokinetic parameters are log-normal in distribution. Hence, a log-transformation was used prior to analysis so that the resulting parameters were approximately normal in their distribution. Since some of the parameters were negative, its absolute value was used for the log-transformation. Possible outliers could then be detected by plotting the principal components against each other [19]. One critical decision is the number of components to retain. Kaiser's rule can be invoked, where only eigenvalues greater than 1 are retained. Another method is to use a Scree plot where the eigenvalues are plotted against their index number. Only those eigenvalues prior to where a change in slope is detected are retained. Zwick & Velicer [20] showed that Kaiser's rule tends to overestimate severely the number of components that should be retained. Scree plots are limited in that a break point is often difficult to detect. Karlis *et al.* [14] proposed a new criterion, and using Monte Carlo simulation, showed it to be superior to that of Kaiser under all conditions. For this reason, we used the method of Karlis *et al.* to select the number of principal components to retain (in this case three). To our knowledge this is the first use of this technique in population analyses. Further research is needed to explore the utility of this method and its limitations. Nevertheless, using this methodology, we identified two observations that appeared to have an effect on the parameter estimates.

In summary, a population model for apomine was developed that characterized its pharmacokinetics in cancer patients and healthy subjects under a variety of different conditions, namely in the fasted and fed states, after single and multiple dose, and in males and females. The only important covariate identified was body weight, which was proportional to the volume of the central compartment. The maximum recommended dose of apomine for studies in healthy subjects in the fasted state is 100 mg twice daily.

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