Safety, tolerability, pharmacokinetics and pharmacodynamics of losmapimod following a single intravenous or oral dose in healthy volunteers

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

Over the last 20 years, inhibition of p38 mitogen-activated protein kinase in numerous preclinical studies of inflammatory conditions (e.g. rheumatoid arthritis, as well as pulmonary and vascular disease) has demonstrated compelling evidence for benefit. Safety/toxicity concerns with translation to humans, in addition to unclear pharmacology, have contributed to limited success in clinical programmes. Losmapimod (a potent p38 mitogen-activated protein kinase inhibitor), provided orally and dosed submaximally, has been safe and well tolerated, but in addition has offered improved vascular benefits in several small clinical studies. These data have permitted consideration for treatment in acute coronary syndrome, which has multiple inflammatory components.

WHAT THIS STUDY ADDS

The pharmacokinetic information available in the literature for losmapimod is sparse, and currently non-existent for intravenous dosing. Elucidation of bioavailability of the oral dose, preliminary safety and tolerability of a single intravenous infusion, along with the comparative pharmacokinetics (PK) and PK/pharmacodynamics (PD) of intravenous and oral dosing are provided herein. Exploration of PK/PD relationships, particularly PK/PD modelling of the relationship between pHSP27 (a monocyte tissue marker) and losmapimod concentrations, is presented with a specific temporal examination. This study permits consideration of an intravenous formulation to be applied during the life-cycle of an acute coronary syndrome development plan.

AIMS

The purpose of this study was to establish safety and tolerability of a single intravenous (IV) infusion of a p38 mitogen-activated protein kinase inhibitor, losmapimod, to obtain therapeutic levels rapidly for a potential acute coronary syndrome indication. Pharmacokinetics (PK) following IV dosing were characterized, and pharmacokinetic/pharmacodynamic (PK/PD) relationships between losmapimod and phosphorylated heat shock protein 27 (pHSP27) and high-sensitivity C-reactive protein were explored.

METHODS

Healthy volunteers received 1 mg losmapimod IV over 15 min ($n = 4$) or 3 mg IV over 15 min followed by a washout period and then 15 mg orally (PO; $n = 12$). Pharmacokinetic parameters were calculated by noncompartmental methods. The PK/PD relationships were explored using modelling and simulation.

RESULTS

There were no deaths, nonfatal serious adverse events or adverse events leading to withdrawal. Headache was the only adverse event reported more than once ($n = 3$ following oral dosing). Following 3 mg IV and 15 mg PO, C_{max} was 59.4 and 45.9 μg l^{-1} and $AUC_{0-\infty}$ was 171.1 and 528.0 μg h l^{-1}, respectively. Absolute oral bioavailability was 0.62 [90% confidence interval (CI) 0.56, 0.68]. Following 3 mg IV and 15 mg PO, maximal reductions in pHSP27 were 44% (95% CI 38%, 50%) and 55% (95% CI 50%, 59%) occurring at 30 min and 4 h, respectively. There was a 17% decrease (95% CI 9%, 24%) in high-sensitivity C-reactive protein 24 h following oral dosing. A direct-link maximal inhibitory effect model related plasma concentrations to pHSP27 concentrations.

CONCLUSIONS

A single IV infusion of losmapimod in healthy volunteers was safe and well tolerated, and may potentially serve as an initial loading dose in acute coronary syndrome as rapid exposure is achieved.

Introduction

Losmapimod (GW856553) acts as a selective p38α/β mitogen-activated protein kinase (p38 MAPK) inhibitor by competitively binding to the ATP binding site of p38MAPK [1]. In preclinical studies of myocardial ischaemia–reperfusion injury, inhibition of p38MAPK has been shown to be cardioprotective. Increased cell viability was noted, with reductions in infarct size, neutrophil accumulation and circulating markers of cell injury, such as creatine kinase and lactate dehydrogenase [2–4]. In angiotensin II-induced atherosclerotic rats, inhibition of p38 MAPK reduced superoxide anion generation with subsequent improvements in endothelial function, mean blood pressure and cardiac hypertrophy [5]. In untreated hypercholesterolaemic patients, losmapimod improved nitric oxide-mediated vasoregulation, a marker of endothelial function, and reduced levels of the inflammatory biomarker high-sensitivity C-reactive protein (hsCRP) [6]. More recently, our group has also demonstrated that losmapimod reduces vascular inflammation as evidenced by fluorodeoxyglucose positron emission tomography-computed tomography in patients with pre-existing atherosclerosis on standard medical therapies, including statins [7]. This body of evidence supports ongoing clinical trials of losmapimod for the indication of acute coronary syndrome (ACS; http://clinicaltrials.gov/ct2/show/NCT00910962).

To date, only oral (PO) doses of losmapimod have been studied. This may not be an ideal formulation in ACS as it takes approximately 1–4 h for losmapimod to reach maximal concentrations. The correlation between a delayed percutaneous coronary intervention and increased risk of various negative health outcomes, including mortality, is generally accepted [8–10]. In particular, preclinical work highlighted that the cardioprotective effects of inhibiting the p38 MAPK pathway are rapidly attenuated after reperfusion [3]. To achieve a rapid onset of action and maximize potential benefits of treatment, an intravenous (IV) formulation of losmapimod has been developed.

The primary purpose of this study was to assess the feasibility of providing a single IV infusion to establish rapid drug exposure. To this end, we evaluated preliminary safety and tolerability, determined the pharmacokinetics (PK) and explored the pharmacodynamics (PD) of an IV formulation of losmapimod. The absolute bioavailability for oral dosing was also determined. Concentrations of phosphorylated heat shock protein 27 (pHSP27) were determined following losmapimod dosing using standard statistical methods and modelling and simulation techniques. Phosphorylated HSP27 was measured because it is a fairly direct downstream phosphorylation target of p38 MAPK. High-sensitivity C-reactive protein was measured because it represents a commonly measured marker of inflammation and plaque vulnerability, and p38 MAPK inhibition has previously been shown to result in hsCRP reductions over 3 days [11]. In future studies, hsCRP may serve some prognostic value to provide better assessment of the risk of experiencing a cardiovascular event [12].

Methods

Study design

This was a Phase 1, open-label, single-centre, multiple-cohort study in healthy adult volunteers (male or female subjects, aged 18–75 years, weighing > 50 kg, body mass index 19–30 kg m^{-2}) that consisted of the following four study periods: screening, treatment, washout (for cohort 3 only) and follow-up. The protocol was approved by an independent ethics committee (Welwyn Clinical Pharmacology Ethics Committee) and was registered at clinicaltrials.gov (NCT01039961). The study complied with the Declaration of Helsinki, and full written informed consent was obtained from all participants before the performance of any study-specific procedures.

Cohort 1 ($n = 4$) received a single 1 mg IV dose of losmapimod as a constant IV infusion over 15 min. Pharmacokinetics results from cohort 1 were sufficient to obviate the need for an optional dose-finding cohort, cohort 2, and were used to determine the dose for cohort 3 predicted to achieve target concentrations. The primary PK parameter for dose selection for cohort 3 was the maximum concentration (C_{max}). Total exposure area under the concentration–time curve (AUC) was also evaluated to ensure that exposure of the IV dose did not exceed exposures previously tested and known to be safe. The C_{max} for cohort 3 was to approach but not exceed the maximal concentration after administration of a single 20 mg oral dose in healthy volunteers, i.e. 73.7 ng ml^{-1} [geometric mean, range 57.8–94.5 ng ml^{-1}, $n = 9$ (data in house)]. A 20 mg single oral dose had previously been shown to be safe and well tolerated and within the dose-linear PK range. Cohort 3 ($n = 12$) received a single 3 mg IV dose of losmapimod infusion over 15 min and, following a 1 week washout period, a 15 mg oral dose of losmapimod given as two 7.5 mg tablets. Subjects fasted for approximately 10 h prior to receiving study drug and received a meal approximately 4 and 10 h postdose. Follow-up occurred 14 ($\pm$3) days following the last dose of study drug.

Safety assessments and safety analysis

All adverse events (AEs)/serious AEs were collected from the time of administration of the investigational product until follow-up. Additionally, serious AEs assessed as related to study participation were recorded from the time the subject gave consent. A complete set of safety observations was performed, including vital signs, physical examination, clinical laboratory evaluations (clinical chemistry, haematology and urinalysis) and 12-lead electrocardiograms (ECGs). The change from baseline was calculated by subtracting the baseline values from the individual postrandomization values. Laboratory, ECG or vital sign values

of potential clinical importance based on predefined criteria were listed for each evaluation.

Pharmacokinetic assessments

Pharmacokinetic samples for determination of losmapimod and GSK198602 (the primary but inactive metabolite) were collected predose and at 5, 10, 15, 30, 45, 60 and 90 min, and 2, 3, 4, 6, 8, 10, 12, 16 and 24 h. Samples were chilled on wet ice immediately after collection into EDTA-containing tubes. Plasma was separated by centrifugation at 3000g, promptly transferred to an appropriately labelled polypropylene tube and frozen at approximately −20°C. The samples were analysed using a validated analytical method based on protein precipitation, followed by HPLC-MS/MS analysis. The lower limit of quantification for losmapimod was 0.2 µg l^{-1} and for GSK198602 it was 1 µg l^{-1} using a 50 µl aliquot of human plasma with a higher limit of quantification of 200 and 1000 µg l^{-1}, respectively. The relative error (calculated using the average experimental concentration for each quality control level) and coefficient of variation (CV) for the quality controls for analysis of losmapimod were ≤10.1 and ≤10.7%, respectively, representing accuracy and precision for the analysis. For GSK198602, the precision and accuracy at a concentration of 3 µg l^{-1} (the low quality control) were ≤53.5 and ≤80.0%. At concentrations of 50 and 800 µg l^{-1} (the middle and high quality controls), the relative error and CV were ≤11.6 and ≤5.3%. Given that the quality control samples were run in duplicate, the run was accepted if no more than one-third of the quality control results exceeded acceptable limits and at least half of the results at each concentration were within the acceptable limit.

Plasma losmapimod and metabolite GSK198602 concentration–time data were analyzed by noncompartmental methods using WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA, USA). From the plasma concentration–time data, the following PK parameters were determined: C_{max}, time to maximum concentration (T_{max}), area under the concentration time curve to last measured concentration (AUC_{0-t}), area under the concentration time curve to infinity ($AUC_{0-\infty}$), half-life ($t_{1/2}$), volume of distribution calculated by the steady-state method (VDss; losmapimod IV dosing only) and clearance (CL) for IV dosing or apparent clearance (CL/F) for oral dosing (losmapimod only). Absolute oral bioavailability was also calculated, and the calculation method is described in the 'Statistical methods' section.

Pharmacodynamic assessments

Phosphorylated HSP27 and hsCRP were collected from subjects in cohort 3 only. Blood samples for measurement of pHSP27 were collected predose and at 30 and 60 min, and 2, 4, 6 and 8 h. One whole blood sample (1 ml) was collected from each subject into a sterile polystyrene tube containing sodium heparin at each time point. Human whole blood was stimulated with sorbitol for 1 h at 37°C in

air enriched with 5% CO_2 or incubated with medium as a control. Sorbitol stimulation induces phosphorylation of HSP27 and serves as a measure of p38 activity. The whole blood samples were lysed on ice after sorbitol stimulation and the lysates frozen. The samples were analysed with a sandwich immunoassay using the Meso Scale Discovery Multi-Spot Biomarker Detection Whole Cell Lysate Kit Phospho-(Ser15)/Total-HSP27 and Meso Scale Discovery SECTOR Instrument (Meso Scale Discovery, Rockville, MD, USA). Details of a similar method are provided elsewhere [13]. The quantifiable range for the assay was 0.78–50 ng ml^{-1}. The absolute relative error (calculated using the average experimental concentration for each quality control level) and CV for the quality controls were ≤8.4 and ≤8.7%, respectively.

To determine hsCRP concentrations, serum samples were collected predose and at 6, 12 and 24 h. Immunonephelometry was used to measure hsCRP on the BN II system (Siemens, Tarrytown, NY, USA) using the Cardio-Phase hsCRP reagent (Siemens). The average accuracy of this method compared with the International Reference Standard CRM470 was ~98%. The CVs at concentrations of 1.73 and 5.1 mg l^{-1} were 6.9 and 7.2%, respectively. The sensitivity of the assay was 0.175 mg l^{-1}, with an upper reporting range of 220 mg l^{-1}.

Statistical methods

Pharmacokinetic analysis For the absolute bioavailability of oral losmapimod, following $\log_e$ transformation, dose-normalized $AUC_{0-\infty}$ values of losmapimod in cohort 3 were analysed using a mixed effects model with a fixed effect term for treatment (IV treatment D or PO treatment E). Subjects were treated as a random effect. A point estimate and associated 90% confidence interval (CI) was constructed for the difference between oral *vs.* IV dosing (i.e. E minus D). The point estimate and associated 90% CIs were then back transformed to provide a point estimate and 90% CI for the ratio E:D (i.e. the absolute bioavailability).

Pharmacodynamic analysis Effects of losmapimod on pHSP27 and hsCRP levels, following $\log_e$ transformation, from cohort 3 were analysed separately using a repeated measure mixed effects model with fixed effect terms for time (baseline and other postdose time points), treatment (IV dosing and oral dosing) and time by treatment interaction. Subject was treated as a random effect in the model. Point estimates at each planned time point and their associated 95% CIs were constructed for the differences of postdose *vs.* baseline for each treatment. They were then back transformed to provide point estimates and 95% CIs for the ratios of postdose to baseline for each treatment at every planned postdose time point.

Pharmacokinetic/pharmacodynamic analysis The relationship between losmapimod concentration and PD end-points (pHSP27 and hsCRP) were explored through

modelling and simulation techniques. Modelling was performed using NONMEM VII (Icon Development Solutions, Ellicott, MD, USA) with the iterative two-stage (ITS) followed by the stochastic approximation expectation maximization (SAEM) estimation methods. The objective function was obtained using the Monte-Carlo importance sampling method assisted by mode *a posteriori* estimation (IMPMAP). The PK/PD models were compared based on visual inspection of the goodness-of-fit plots, a successful covariance step, and a significant change of the model selection criteria (i.e. the Akaike information criteria). Two PK/PD models were evaluated, the direct-link and indirect maximal inhibitory effect (I_{max}) models. The PD data were normalized to baseline and then converted to a symmetrized percentage change to create a normal distribution of the data for modelling [14]. For plots, data were back transformed to the robust change from baseline for easier interpretation. The PK/PD model was evaluated by visual predictive check ($n = 1000$ per dose).

Results

Study population

A total of 16 healthy subjects were enrolled in the study (four in cohort 1 and 12 in cohort 3). Demographic data are provided in Table 1.

Adverse events

There were few adverse events reported during the study. The only AE reported in more than one subject was headache, which was reported in three subjects receiving a 15 mg oral dose. In the 15 mg oral dosing arm, eye pain, nasopharyngitis and contusion were also reported as AEs. In the 1 mg IV arm, headache and thirst were reported once each. In the 3 mg IV arm, headache, neuralgia, catheter site haematoma, fatigue, dry mouth, nausea (the only drug-related AE determined by the investigator) and nasopharyngitis were reported once each. No subject died,

experienced a serious AE or withdrew due to an AE during the study. There were no clinically significant alterations in vital signs, ECG, haematology, biochemistry and urinalysis.

Pharmacokinetic results

Mean losmapimod concentration–time profiles for the three doses are presented in Figure 1. The PK parameters obtained from the noncompartmental analysis for losmapimod and GSK198602 are presented in Table 2. Plasma concentrations for the 3 mg IV dose reached the target, i.e. they approached but did not exceed the C_{max} of a 20 mg oral dose. Comparatively, the 1 mg IV dose did not reach this concentration. Absolute oral bioavailability was 0.62 (90% CI 0.56, 0.68).

Pharmacodynamic results

The postdose/baseline ratio at each time point, i.e. the change from baseline, for both biomarkers are presented in Table 3. Following IV dosing, the concentration of pHSP27 was reduced from baseline by 44% within 30 min of administration, was maintained at approximately the same level for 1 h, and then increased almost completely back to baseline by 8 h (Figure 2). Following oral dosing, the level of pHSP27 was decreased by 33% at 1 h, 55% at 4 h (T_{max} for losmapimod), and diminished thereafter (Figure 2).

There was a 17% reduction for hsCRP at 24 h in the oral treatment arm; however, a significant reduction in hsCRP was not seen at any other time point following 15 mg PO or at any time point following 3 mg IV dosing.
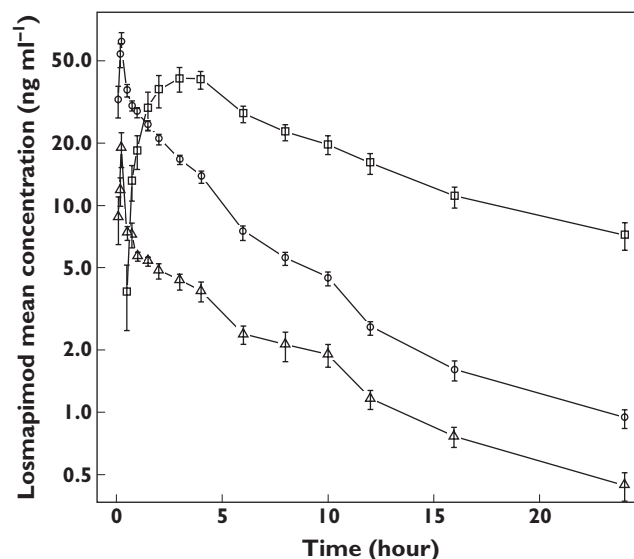
Table 1

Patient demographics

Demographics	1 mg IV (n = 4)	3 mg IV/15 mg PO (n = 12)
Age [years; mean (SD)]	32.3 (8.54)	43.3 (9.25)
Body mass index [kg m^{-2}; mean (SD)]	24.67 (2.01)	25.00 (2.55)
Height [cm; mean (SD)]	180.3 (5.56)	175.4 (6.23)
Weight [kg; mean (SD)]	79.96 (3.68)	76.77 (7.13)
Male sex [n (%)]	4 (100)	12 (100)
Race [n (%)]		
African American/African heritage	0	1 (8)
White, Arabic/North African heritage	1 (25)	0
White, White/Caucasian/European heritage	3 (75)	11 (92)

Figure 1

Mean concentration at each time point with standard error bars. □, 15 mg orally (PO); ○, 3 mg intravenously (IV); and △, 1 mg IV

Table 2

Results of noncompartmental analysis for losmapimod and the primary metabolite GSK198602

Parameter	Compound	Dose	Geometric mean	95% CI	CV%
$AUC_{0-\infty}$ (μg h l^{-1})	Losmapimod	1 mg IV	54.09	38.4, 76.2	21.8
		3 mg IV	171.1	148, 198	23.3
		15 mg PO	528.0	428, 652	34.1
	GSK198602	1 mg IV	30.67	23.5, 40.0	16.8
		3 mg IV	216.5	178, 263	31.2
		15 mg PO	1096	875, 1372	36.5
AUC_{0-t} (μg h l^{-1})	Losmapimod	1 mg IV	48.56	36.2, 65.1	18.6
		3 mg IV	161.7	141, 186	22.1
		15 mg PO	421.0	351, 506	29.5
	GSK198602	1 mg IV	22.42	14.6, 34.5	27.5
		3 mg IV	184.8	155, 221	28.4
		15 mg PO	717.9	616, 837	24.4
C_{max} (μg l^{-1})	Losmapimod	1 mg IV	18.00	8.91, 36.3	46.4
		3 mg IV	59.38	46.1, 76.5	41.5
		15 mg PO	45.90	36.7, 57.4	36.3
	GSK198602	1 mg IV	5.228	4.01, 6.81	16.7
		3 mg IV	22.24	20.1, 24.6	16.1
		15 mg PO	67.31	55.5, 81.6	30.9
T_{max} (h)*	Losmapimod	1 mg IV	0.250	0.17, 0.27	—
		3 mg IV	0.250	0.18, 0.75	—
		15 mg PO	3.500	2.00, 6.00	—
	GSK198602	1 mg IV	1.000	0.75, 1.50	—
		3 mg IV	1.515	1.00, 6.00	—
		15 mg PO	4.000	2.00, 10.00	—
$t_{1/2}$ (h)	Losmapimod	1 mg IV	8.328	4.84, 14.3	35.2
		3 mg IV	6.488	5.14, 8.20	38.1
		15 mg PO	9.439	7.37, 12.1	40.6
	GSK198602	1 mg IV	4.014	2.96, 5.44	19.2
		3 mg IV	7.312	5.83, 9.18	36.9
		15 mg PO	12.17	16.4, 0.474	50.2
VDss (l)	Losmapimod	1 mg IV	171.8	139, 212	13.3
		3 mg IV	110.8	96.1, 128	22.7
CL (l h^{-1}) CL/F (l h^{-1})	Losmapimod	1 mg IV	18.49	13.1, 26.0	21.8
		3 mg IV	17.54	15.2, 20.3	23.3
		15 mg PO	28.41	23.0, 35.1	34.1

*T_{max} is presented as the median and range.

Pharmacokinetic/pharmacodynamic analysis

The direct-link I_{max} model was selected to describe the effect between pHSP27 concentrations and losmapimod plasma concentrations as follows:

$$E(t) = E_0 - \frac{I_{max} \times C(t)}{IC_{50} + C(t)}$$

where E_0 is the effect at baseline (which was fixed to 1 because the data were normalized to baseline), $E(t)$ is the effect observed at time t (i.e. the fraction of baseline pHSP27 at time t), I_{max} is the maximal inhibitory effect achievable, which was fixed to 0.667 (equivalent to a robust maximal change from baseline of 0.8 or 80% and based on previous data), $C(t)$ is the plasma concentration of losmapimod at time t, and IC_{50} is the losmapimod concentration which results in half-maximal inhibition of the phosphorylation of HSP27. The only parameters estimated

in this model were the IC_{50}, the interindividual variability of the IC_{50} (CV%) and the residual error (additive), which were 37.4 ng ml^{-1} (9.83% relative standard error), 31.8% (32.1% relative standard error) and 0.0044 (26.8% relative standard error), respectively. The visual predictive check for this model, depicted as the percentage change from baseline to display the inhibitory effect more clearly, is presented in Figure 2. Essentially, this model demonstrates that the inhibition of phosphorylation of HSP27 is directly related to the plasma concentration of losmapimod and as the losmapimod concentration changes with time the resulting change in pHSP27 occurs almost instantaneously.

Discussion

Losmapimod, as a single IV dose (given as a 15 min infusion) administered to a small cohort of healthy volunteers, was well tolerated with an adverse event profile similar to

Table 3

Parametric analysis for the comparison of phosphorylated heat shock protein 27 (pHSP27) and high-sensitivity C-reactive protein (hsCRP) levels between postdose and baseline (i.e. ratio of postdose/baseline)

Parameter	Formulation	Time	Point estimate (95% confidence interval)	P Value
pHSP27	IV	30 min	0.56 (0.50, 0.62)	<0.0001
	IV	60 min	0.56 (0.51, 0.63)	<0.0001
	IV	2 h	0.65 (0.59, 0.73)	<0.0001
	IV	4 h	0.70 (0.63, 0.78)	<0.0001
	IV	6 h	0.84 (0.76, 0.94)	0.0022
	IV	8 h	0.89 (0.80, 0.99)	0.0366
	PO	30 min	0.96 (0.87, 1.07)	0.5081
	PO	60 min	0.67 (0.69, 0.74)	<0.0001
	PO	2 h	0.55 (0.49, 0.61)	<0.0001
	PO	4 h	0.45 (0.41, 0.50)	<0.0001
	PO	6 h	0.51 (0.46, 0.57)	<0.0001
	PO	8 h	0.54 (0.48, 0.60)	<0.0001
hsCRP	IV	6 h	1.00 (0.91, 1,11)	0.9257
	IV	12 h	1.01 (0.92, 1.12)	0.7888
	IV	24 h	0.95 (0.87,1.05)	0.3393
	PO	6 h	0.98 (0.89, 1.08)	0.7396
	PO	12 h	0.95 (0.86, 1.05)	0.3072
	PO	24 h	0.83 (0.76, 0.91)	0.0004

that shown previously [6]. This outcome was expected because exposures following IV dosing aligned with plasma concentrations previously shown to be generally safe and well tolerated in a larger number of subjects.

Losmapimod delivered as a 15 min IV infusion displayed approximate dose proportionality between the 1 and 3 mg doses. The volume of distribution at steady state of 171.8 l for the 1 mg IV dose and 110.8 l for the 3 mg IV dose indicates that this drug distributes extensively into tissues. There was a statistically significant difference in the VDss between these two doses. Elucidating the basis for this difference is difficult because there was a limited number of subjects, particularly in the 1 mg dose group ($n = 4$), and the ranges for VDss of the two doses overlapped; 140.9–186.0 l for the 1 mg dose and 76.8–153.2 l for 3 mg dose. However, one possible explanation may be due to covariates, particularly body weight, because bodyweight was found to be a significant covariate on clearance and volume of distribution terms in a population PK analysis in ACS patients (unpublished data on file; GlaxoSmithKline, King of Prussia, PA, USA). With regard to this study, the two patients who had the lowest bodyweights, i.e. 66.4 and 66.8 kg, received the 3 mg IV dose and had the lowest VDss, i.e. 76.8 and 86.1 l.

This is the only study involving the IV formulation of losmapimod thus far. However, numerous other phase I studies in healthy volunteers have been conducted utilizing a tablet formulation. The PK from those studies closely parallels our present findings. The half-life in this study ranged between 6.5 and 9.4 h (based on the geometric mean for each dose group), while the half-life in the first time in human (FTIH) escalating dose study ranged between 4.4 and 10.1 h for doses in the range of 7–60 mg (unpublished data on file; GlaxoSmithKline, King of Prussia, PA, USA). The T_{max} for the oral dose in this study was also in good agreement with the T_{max} of the 20 mg single dose in the previous FTIH study; 3.5 h (median), range 2–6 h compared with 3.0 h (median), range 1.5–4 h, respectively. Finally, the apparent clearance in the present study for the oral dose, 28.4 l h^{-1}, also appeared to be similar to that for the 20 mg single dose in the FTIH study, 33.3 l h^{-1}.

The PK/PD relationship between losmapimod and pHSP27, a fairly direct monocyte marker [15] within the p38 phosphorylation cascade, was characterized using a direct-link standard I_{max} model. This model demonstrated that the pharmacodynamic effect of losmapimod on this biomarker occurs instantaneously and correlates directly with losmapimod plasma concentrations, demonstrating that an IV infusion over a short duration of time achieves a more rapid inhibition of p38 MAPK compared with the oral dose. As noted, this may be a desirable effect in ACS. In contrast, hsCRP did not reveal such a PK/PD relationship (Figure 3). The most likely explanation as to why an exposure–response relationship for hsCRP could not be developed with these data is the long half-life of CRP (19–20 h [16, 17]). Several consecutive days of dosing would be needed in order to observe the maximal effect of losmapimod on hsCRP. Another major contributory factor is the fact that healthy volunteers were used in this study, with low baseline hsCRP levels (geometric mean baseline hsCRP 0.6 and 0.55 mg l^{-1} for the 3 mg IV and 15 mg oral dose, respectively) compared with the higher hsCRP levels typically observed in a population with coronary disease. Other possible explanations include high variability of hsCRP and small sample size of this study. However, despite this rationalization as to why an exposure–response relationship was not observed, a trend of reduced hsCRP was noted for both formulations at 24 h (which was statistically significant for the PO formulation) and for only the PO formulation at 12 h.

Conclusion

A single IV infusion of losmapimod in healthy volunteers is safe and well tolerated and, based on its effect on pHSP27, demonstrates rapid blockade of the p38 MAPK receptor. These data suggest that an initial IV loading dose of losmapimod may be of potential benefit in acute coronary syndrome.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). A.T.M. and J.G. declare no support from any organization for the submitted work; no financial relationships with any organiza-
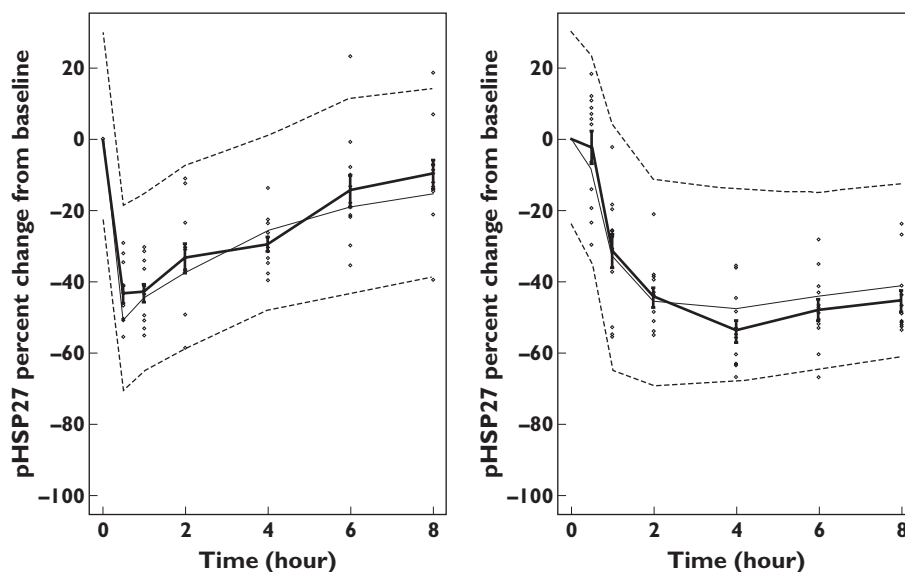
Figure 2

Visual predictive check forphosphorylated heat shock protein 27 (pHSP27) direct inhibitory model with observed data. Mean of observed data at each time point with standard error bars and thick continuous line. The median of simulated data at each time point is shown by the thin continuous line. The 90% prediction interval is indicated by the thin dashed lines. ◇, observed data. Left panel shows data for 3 mg IV and right panel 15 mg PO. The model appears to describe the central tendency and variability of the data well, supporting the suitability of the model
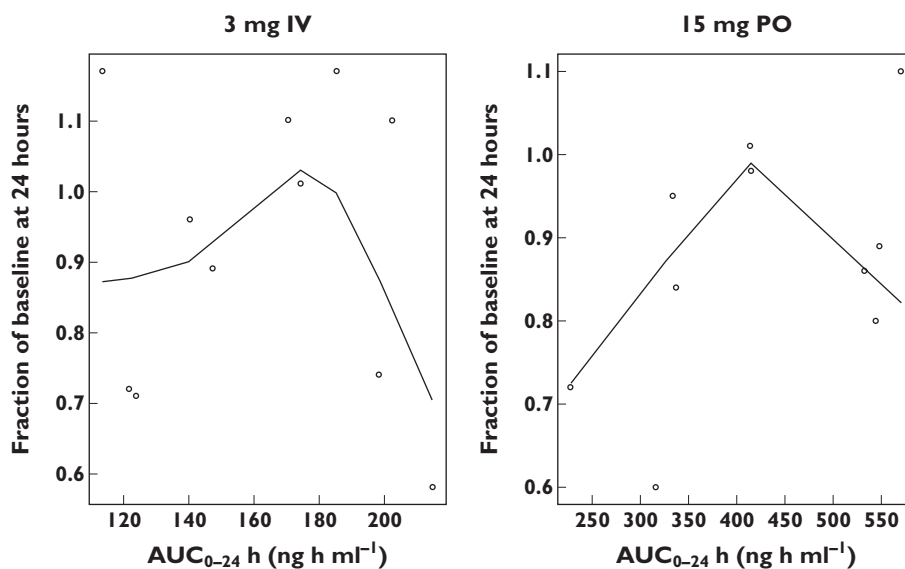
Figure 3

AUC_{0-24} *vs.* change from baseline high-sensitivity C-reactive protein (hsCRP) with smooth (i.e. a lowess fit curve). No exposure–response relationship was demonstrated. The hsCRP values were below the limit of quantification for one subject following 3 mg IV dosing and two subjects following 15 mg PO dosing

tions that might have an interest in the submitted work in the previous 3 years; and no other relationships or activities that could appear to have influenced the submitted work. A.M.B., L.S.-B., G.C., M.J.F., J.M. and D.L.S. had support from GlaxoSmithKline in the form of employment as A.M.B., L.S.-B., G.C., M.J.F., J.M. and D.L.S. are full-time

employees of GlaxoSmithKline. A.M.B., L.S.-B., G.C., M.J.F., J.M. and D.L.S. also own stock in GlaxoSmithKline. D.L.S. declares that he led the full clinical discovery for losmapimod, which could appear to have influenced the submitted work. J.C. declares that he is a full-time employee of Cambridge University Hospitals NHS Foundation Trust but

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