Prolonged pharmacodynamic effects of S-0139, an intravenously administered endothelin A (ET_A) antagonist, in the human forearm blood flow model

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Using the technique of venous occlusion plethysmography, endothelin (ET) antagonists have been shown to block the vasoconstrictive effects of intra-brachial ET-1 infusion on forearm blood flow.
- Effects have been reported only when significant plasma concentrations of the antagonists are present.

WHAT THIS STUDY ADDS

- S-0139 is a selective ET antagonist given by intravenous administration.
- In this study it is shown to have a prolonged duration of pharmacodynamic activity beyond that expected from its pharmacokinetic profile.

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Keywords

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AIMS

To estimate the pharmacologically active dose range of a new investigational compound S-0139, a selective endothelin A (ET_A) receptor antagonist, in man, and to examine the duration of its pharmacodynamic effect.

METHODS

Venous occlusion plethysmography was performed to assess changes in forearm blood flow following intra-brachial administration of endothelin-1 (ET-1). ET_A antagonists have been shown to block ET-1-induced vasoconstriction in this model. The study was conducted in three parts: (1) a pilot study to explore dose-response (dose range 0.08–13.33 µg kg⁻¹ min⁻¹), (2) a randomized study to confirm dose-response (placebo, 2.5, 6.67 and 15 µg kg⁻¹ min⁻¹), and (3) a delayed administration study (15.7 µg kg⁻¹ min⁻¹) to explore the duration of the pharmacodynamic effect. In all studies a 3-h infusion of S-0139 was given and during the last 90 min of the infusion, ET-1 was infused concurrently for 90 min. In study (3) a second ET-1 infusion was given starting 3 h after completion of the first.

RESULTS

Intravenously administered S-0139 resulted in significant inhibition of ET-1-induced vasoconstriction in the forearm (plasma concentration 800–2000 ng ml⁻¹). In the delayed administration study, the same extent of inhibition was still present when ET-1 was administered 3 h after the end of infusion of S-0139, even though the S-0139 plasma concentrations (mean 17 ng ml⁻¹) were well below pharmacologically active concentrations as determined in studies 1 and 2.

CONCLUSIONS

S-0139 dose-dependently blocks ET-1-mediated vasoconstriction in the forearm and has a prolonged duration of effect beyond that expected from its pharmacokinetic profile.

Introduction

Endothelin-1 (ET-1) is one of the most powerful vasoconstrictors known. ET receptor antagonists have therefore been suggested as potential therapy for conditions with raised peripheral vascular resistance such as heart failure and hypertension [1]. Both non-selective and selective antagonists have been evaluated in heart failure, but in general were associated with worsening of the condition [2, 3]. However, the selective endothelin A (ET_A) antagonist sitaxentan and the non-selective antagonist bosentan have received approval for the treatment of primary pulmonary hypertension, and, recently, the selective antagonist, clazosentan, has been shown to have efficacy in preventing vasospasm following subarachnoid haemorrhage [4]. An alternative indication where antagonizing the vasoconstrictive action of endothelin may be of benefit is ischaemic stroke. The investigational compound S-0139 is a new potent (K_i 1 nM) and ET_A-selective (700-fold) receptor antagonist [5]. It is effective in rat [6] and cat [7] middle cerebral artery occlusion models in increasing cerebral blood flow and reducing lesion size, brain oedema and mortality.

The rationale for the choice of doses and dosing regimen for clinical stroke outcome studies is a vexed question. In order to provide preliminary data concerning likely beneficial doses for S-0139, the present study used the human forearm circulation as a surrogate resistance bed *en lieu* of the brain. Assessment of forearm blood flow (FBF) is an established methodology, and in general the forearm response predicts systemic effects [8]. Whilst the data supporting direct extrapolation to the brain vasculature is limited, this model allows *in vivo* assessment of the pharmacodynamic effects of S-0139 in humans. The effects of ET antagonists have been previously studied using intra-brachial artery infusion of ET-1 and assessing changes in FBF. TAK-044, a mixed ET_{A/B} antagonist, given intravenously completely blocks the vasoconstriction (reduction of FBF) caused by local administration of ET-1 [9]. Co-administration of BQ-123, a selective ET_A antagonist, with ET-1, both infused intra-arterially, abolished ET-1-induced vasoconstriction [10]. A study of ABT-627, a selective ET_A antagonist, given orally blocked ET-1-induced vasoconstriction, reduced peripheral resistance and had only a mild effect on blood pressure [11].

In addition to exploring the pharmacologically active dose range in this model, the present study aimed to explore the duration of the pharmacodynamic effect to help guide selection of a dosing regimen. S-0139 disappears rapidly from the plasma compartment, with concentrations falling over one order of magnitude within 0.5 h.

However, preclinical results suggested that a prolonged effect of S-0139 is possible: S-0139 has a slow dissociation rate from the ET_A receptor [12] and intermittent administration of S-0139 in an animal stroke model

was shown to be effective (Shionogi, unpublished observation).

Methods

The studies described were conducted in accordance with the Declaration of Helsinki; permission to conduct the studies was obtained from the Local Research Ethics Committee, UK. Written informed consent was obtained from all subjects prior to study start. All studies were carried out in the Clinical Pharmacology Unit of Addenbrooke's Hospital and the GSK Clinical Unit Cambridge, UK. The study was sponsored by Shionogi-GlaxoSmithKline LLC.

Subjects

Healthy male subjects, aged 18–55 years, of body weight >50 kg with a body mass index of 19–29.9 kg m⁻² were recruited.

Study design

The results in this publication represent data from three consecutive studies, summarized in Figure 1. During all studies ET-1 was infused for the final 90 min of a 3-h infusion of S-0139.

- 1 Pilot Study** – A single blind pilot study in five subjects to provide preliminary dose–response data for S-0139 in the FBF model. The subjects were fully blinded. Six active doses of S-0139 were investigated, ranging from 0.08 to 13.33 µg kg⁻¹ min⁻¹. Individual subjects received a maximum of three active doses and placebo using an incomplete block design. There was at least 6 days between dosing.
- 2 Dose–response study** – S-0139 was evaluated in a double-blind, randomized, placebo-controlled crossover study to assess the effect of three active dose levels – 2.5, 6.67 and 15 µg kg⁻¹ min⁻¹ and placebo. Eleven subjects each received up to three active doses and placebo. Subjects nominally participated in four dosing sessions; however, one session could be repeated if it was halted prematurely due to problems with brachial cannulation. There was at least 6 days between dosing.
- 3 Delayed administration study** – This was a double-blind, randomized, placebo-controlled, parallel-group study. Subjects participated in one session only and were randomized to receive either S-0139 or placebo. In comparison with studies 1 and 2, subjects received a second ET-1 infusion starting 3 h after completion of the first. S-0139 was administered at a dose of 15.7 µg kg⁻¹ min⁻¹. A total of 19 subjects (13 active, six placebo) were included in this study.

Forearm blood flow

FBF was assessed in both arms using venous occlusion plethysmography with mercury-in-silastic strain gauges,

Study 1:

Pilot, N = 5 subjects

Single blind, randomised, placebo controlled ascending dose study

Study 2:

Dose response, N = 11 subjects

Double blind, randomised, placebo controlled study

Study 3:

Delayed administration, N = 19 subjects

Double blind, randomised, placebo controlled study

FBF measurements were made every 15 min during the ET-1 infusions

Figure 1

Schematic representation of the study designs

which measured the increase in forearm volume, following established procedures [8]. FBF was measured for 3-min periods at 15-min intervals during the ET-1 infusions; the mean of the final five measurements over each 3-min period was used for analysis.

Systemic haemodynamics

Supine blood pressure (BP) and heart rate (HR) were measured with an Omron 705CP; cardiac output was measured with an EXT-TEBCO (External Thoracic Electrical Bioimpedance Cardiac Output) module. For the pilot and dose-

response FBF studies, measurements of BP and HR were made at baseline and thereafter every 15 min during the study. For the delayed administration study, measurements were made at baseline prior to drug administration and then every 30 min during both the 3-h infusion of S-0139 and during the second ET-1 infusion.

Cardiac index was calculated from cardiac output divided by body surface area. In studies 1 and 2 total peripheral vascular resistance index (TPRI) was calculated as (mean arterial pressure minus central venous pressure) divided by cardiac index. A predefined value for central venous pressure of 4 mmHg was used. In study 3 TPRI was calculated from mean arterial pressure divided by cardiac index.

Study procedures

Testing took place in a quiet, temperature-controlled ($23 \pm 2^\circ\text{C}$) research laboratory. A venous cannula was placed into the antecubital fossa of each arm, one for infusion of S-0139 and one for collections of blood samples for estimation of S-0139, ET-1 and safety laboratory assessments. For the pilot and dose-response studies a 27-G unmounted steel needle was inserted into the brachial artery (non-dominant arm) under local anaesthesia (1% lignocaine hydrochloride). For the delayed administration study, a 20-G arterial cannula was inserted into the brachial artery (non-dominant arm) under local anaesthesia to allow a second ET-1 infusion. In all studies ET-1 was infused intra-arterially at a rate of 5 pmol min^{-1} . A pilot study conducted prior to the third study confirmed the consistency of the desired FBF response for ET-1 at 5 pmol min^{-1} , when given as two intra-arterial administrations separated by 3 h.

Serial blood samples were taken for estimation of plasma S-0139 and systemic levels of ET-1. Samples were analysed for S-0139 by protein precipitation with high-performance liquid chromatography-tandem mass spectrometry (lower limit quantification 2 ng ml^{-1}), with precision coefficient of variation (% CV) of $\leq 10.8\%$ within assay and $\leq 11.1\%$ between assay and assay accuracy of $-11.5\% \leq \text{bias} \leq 10.9\%$. ET-1 was analysed using a commercially available enzyme-linked immunosorbent assay kit from R&D Systems (Minneapolis, MN, USA).

Study drugs

S-0139 was supplied in vials containing the di-sodium salt (300 mg, equivalent to 283 mg of the free acid), which was reconstituted using 10 ml 0.9% saline. An appropriate amount of this concentrated solution was used to give the desired dose according to the person's body weight and further diluted with 0.9% saline. The total infusate volume administered was 500 ml in studies 1 and 2 and 250 ml in study 3.

ET-1 supplied by ClinAlfa (Bachem AG, Bubendorf, Switzerland) was used for studies 1 and 2 and from an internal GSK supply for study 3. It was supplied as vials containing

sterile, white, lyophilized powder for reconstitution. The lyophilized powder was reconstituted using 0.9% saline and was then diluted to the required concentration. It was infused at a rate of 1 ml min^{-1} (5 pmol min^{-1}).

Data analysis and statistical considerations

The following parameters were calculated for each ET-1 infusion for each subject: FBF response, calculated as $\text{FBF ratio} = \text{FBF of infused arm} / \text{FBF non-infused arm}$; the percent change in FBF response = $(\text{FBF}_t - \text{FBF}_0) / \text{FBF}_0 \times 100$, where FBF_t was the FBF ratio at time t after the ET-1 infusion, and FBF_0 was the FBF ratio before the ET-1 infusion; and area under the FBF% response time curve from baseline over the duration of the ET-1 infusion (AUEC_{0-90}), calculated using the trapezoidal rule [between time = 1.5 h (start of ET-1 infusion) and 3 h (end of ET-1 infusion)].

For the dose-response study, a mixed effect ANOVA model was fitted to the AUEC_{0-90} . Terms fitted in the model included subject (random) and treatment. Point estimates and 95% confidence intervals (CIs) for the differences 'S-0139 (low dose) vs. placebo', 'S-0139 (medium dose) vs. placebo' and 'S-0139 (high dose) vs. placebo' were constructed using the appropriate variance term. For the delayed administration study, a mixed-effect ANOVA model was fitted to the primary end-point (AUEC_{0-90}). Terms fitted in the model included subject (random), period (first and second ET-1 administration), treatment and treatment-period interaction. Point estimates and 95% CIs for the differences 'S-0139 vs. placebo (first ET-1 admin)', 'S-0139 vs. placebo (second ET-1 admin)' and 'S-0139 vs. placebo (second ET-1 admin) vs. S-0139 vs. placebo (first ET-1 admin)' were constructed using the appropriate variance term. For all analyses, distributional assumptions underlying the analyses were assessed.

Pharmacokinetic parameters were calculated using non-compartmental analyses with WinNonlin version 4.1.

Results

Demographics

The demographic characteristics of the study populations are summarized in Table 1. Five subjects participated in study 1 the pilot study, one subject was withdrawn due to

Table 1

Demography mean ($\pm$ SD) of study subjects (range for age)

	Study		
	Pilot ($n = 5$)	Dose response ($n = 11$)	Delayed administration ($n = 19$)
Age (years)	35 (27–41)	34 (26–45)	37 (20–55)
Height (m)	1.80 (0.06)	1.79 (0.06)	1.80 (0.07)
Weight (kg)	87.2 (10.1)	83.1 (8.1)	79.7 (11.5)

Figure 2
Study 1 – pilot study: mean ($\pm$ SE) plot of % change from baseline in forearm blood flow over time following dosing with S-0139 and placebo. Placebo ($\blacklozenge$); $0.08\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ ($\blacksquare$); $0.25\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ ($\blacktriangle$); $0.83\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ ($\times$); $2.96\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ ($\ast$); $5.92\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ ($\bullet$); $13.33\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ (+)

an adverse event (AE). In study 2, the dose–response study, five subjects completed four sessions; three subjects completed three sessions; and three subjects completed only one session. Two subjects were withdrawn due to AEs and four after the study was halted due to problems with the supply of ET-1. Nineteen subjects participated in study 3, the delayed administration study, 13 received S-0139 and six placebo. Two subjects from the S-0139 group were withdrawn, one due to an AE and one due to problems with the infusion site.

Study 1 – pilot study

The mean percentage change in FBF data from the pilot study are shown in Figure 2. Doses of S-0139 from $0.83\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ were successful in partly inhibiting or completely blocking ET-1-mediated vasoconstriction.

Based upon study 1 results, S-0139 doses of 2.5, 6.67 and $15\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ were evaluated in study 2, the dose–response study.

Study 2 – dose–response study

The percentage change in FBF data from the dose–response study is presented in Table 2 and Figure 3.

Although there were some individual variations, the response to ET-1 infusion with concurrent administration of placebo saw a decrease in FBF of 30–40% from 30 min onwards.

The low dose level of S-0139 ($2.5\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) partially inhibited the ET-1-mediated vasoconstriction. The difference in response to ET-1 (AUEC_{0-90}) between low dose level of S-0139 (-1330) and placebo (-2234) was on average 905 (% change $\times$ min). This difference was not statistically significant ($P = 0.13$, 95% CI -303.6 , 2113.2).

In contrast, the medium ($6.67\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) and high ($15\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) dose levels of S-0139 significantly inhib-

Table 2
Study 1 – FBF response – dose–response study: mean (standard deviation) ratio of infused to non-infused arm and % change from baseline

Regimen	Time (min)	N	Ratio infused: non-infused arm	% Change from baseline
Placebo	0	7	1.25 (0.39)	
	15	7	1.00 (0.49)	–22.27 (18.38)
	30	7	0.87 (0.58)	–34.26 (28.96)
	45	7	0.92 (0.49)	–29.01 (24.76)
	60	7	0.97 (0.41)	–25.15 (16.33)
	75	7	0.99 (0.50)	–24.97 (20.90)
	90	7	1.04 (0.59)	–22.60 (26.18)
$2.5\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$	0	7	1.29 (0.52)	
	15	7	1.12 (0.34)	–9.33 (20.39)
	30	7	1.08 (0.42)	–15.36 (16.65)
	45	7	1.08 (0.44)	–16.04 (13.01)
	60	7	1.05 (0.44)	–18.37 (16.61)
	75	7	1.02 (0.39)	–18.89 (22.29)
	90	7	0.97 (0.42)	–24.10 (22.09)
$6.67\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$	0	8	0.97 (0.36)	
	15	8	0.96 (0.26)	2.34 (21.16)
	30	8	0.98 (0.45)	0.05 (18.73)
	45	8	0.94 (0.35)	–1.90 (20.71)
	60	8	0.90 (0.31)	–4.67 (22.83)
	75	8	0.87 (0.30)	–9.02 (21.83)
	90	8	0.97 (0.40)	0.41 (25.45)
$15.0\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$	0	7	0.99 (0.25)	
	15	7	0.98 (0.26)	–0.72 (14.88)
	30	7	0.96 (0.22)	–1.81 (7.66)
	45	7	1.03 (0.32)	3.35 (20.08)
	60	7	0.83 (0.21)	–14.33 (20.87)
	75	7	0.88 (0.17)	–8.35 (23.10)
	90	7	0.81 (0.19)	–16.43 (17.74)

Figure 3

Study 2 – dose–response study: mean ($\pm$ SE) plot of percent change from baseline in forearm blood flow over time following dosing with S-0139 and placebo ($\pm$ SE). $AUEC_{0-90}$ S-0139 $2.5 \mu\text{g kg}^{-1} \text{min}^{-1}$ was not significantly different from placebo ($P = 0.13$). $AUEC_{0-90}$ S-0139 $6.67 \mu\text{g kg}^{-1} \text{min}^{-1}$ and $15 \mu\text{g kg}^{-1} \text{min}^{-1}$ significantly inhibited ET-1-mediated vasoconstriction compared with placebo ($P = 0.0017$ and $P = 0.0024$, respectively). Placebo ($-\diamond-$); $2.50 (\mu\text{g kg}^{-1} \text{min}^{-1})$ ($-\blacksquare-$); $6.67 (\mu\text{g kg}^{-1} \text{min}^{-1})$ ($-\blacktriangle-$); $15.0 (\mu\text{g kg}^{-1} \text{min}^{-1})$ ($-\bullet-$)

Table 3

Study 2 – dose–response study: pharmacokinetic parameters (geometric mean and range) for S-0139

	Dose S-0139 ($\mu\text{g kg}^{-1} \text{min}^{-1}$) 2.5 ($n = 7$)	6.67 ($n = 8$)	15 ($n = 4$)
$AUC_{0-\infty}$ ($\text{ng h}^{-1} \text{ml}^{-1}$)	904 (756–1122)	2560 (2177–3154)	5597 (4509–6725)
C_{max} (ng ml^{-1})	343 (275–426)	1002 (858–1190)	2193 (1950–2390)
CL ($\text{l h}^{-1} \text{kg}^{-1}$)	0.47 (0.38–0.56)	0.44 (0.36–0.52)	0.46 (0.38–0.56)
C_{ss} (ng ml^{-1})	302 (252–374)	853 (726–1051)	1866 (1503–2242)

ited ET-1-mediated vasoconstriction. The difference from placebo in response to ET-1 ($AUEC_{0-90}$) between the medium and high dose level (S-0139) was statistically significant, on average 2032 ($P = 0.0017$, 95% CI 872.4, 3191.4) and 2029 ($P = 0.0024$, 95% CI 820.2, 3237.6) (% change $\times$ min).

Small increases in ET-1 level were measured during the study: from 1.2 to 2.2 pg ml^{-1} at the highest dose of S-0139 comparison with an increase of 0.8 – 1.5 pg ml^{-1} on placebo.

Pharmacokinetic data for S-0139 from the dose–response study are summarized in Table 3. Following the start of intravenous infusion, steady state was achieved within 30 min, and concentrations remained at steady state for the duration of the infusion. After the infusion was stopped the concentrations decreased an order of magnitude within 0.5 h. Over the range tested the exposures were linear with respect to dose and between-subject variability was low with CV of $<20\%$. S-0139 was effective in reversing ET-1-induced vasoconstriction at doses of 6.67

and $15 \mu\text{g kg}^{-1} \text{min}^{-1}$ with corresponding plasma concentrations of approximately 800 – 2000 ng ml^{-1} .

Study 3 – Delayed administration study

In the placebo treatment group a decrease in FBF of 40 – 50% was seen 45 min after the start of the ET-1 infusion. Administration of S-0139 in this study did not fully block ET-1-mediated vasoconstriction (Table 4; Figure 4). However, the attenuation by S-0139 of the FBF response for a second 90-min ET-1 challenge, starting 3 h after the end of S-0139 infusion, was almost identical to when the ET-1 challenge was given during infusion of the ET antagonist.

Following the first ET-1 administration, a smaller decrease in FBF ($AUEC_{0-90}$) was seen for S-0139 (-1295) compared with placebo (-3189). This difference, on average 1894 (% change $\times$ min), was found to be statistically significant ($P = 0.005$, 95% CI 618, 3170). A similar statistically significant difference in FBF response of 1770

Table 4

Study 3 – FBF response – delayed administration study: mean (standard deviation) ratio of infused to non-infused arm and % change from baseline

Regimen	Time	ET-1 time	N	n	Ratio infused : non-infused	% change from baseline
Placebo	1 h 25 min	1st ET-1 pre dose 1	6	6	0.960 (0.27)	
	1 h 30 min	1st ET-1 pre dose 2	6	6	0.993 (0.32)	
	1 h 45 min	1st ET-1	6	6	0.695 (0.15)	–26.518 (16.26)
	2 h	1st ET-1	6	6	0.615 (0.16)	–32.923 (24.28)
	2 h 15 min	1st ET-1	6	6	0.455 (0.12)	–48.050 (26.30)
	2 h 30 min	1st ET-1	6	6	0.487 (0.11)	–45.752 (21.66)
	2 h 45 min	1st ET-1	6	6	0.472 (0.19)	–48.017 (26.54)
	3 h	1st ET-1	6	5	0.520 (0.22)	–42.512 (18.77)
	5 h 45 min	2nd ET-1 pre dose 1	6	6	0.753 (0.39)	
	6 h	2nd ET-1 pre dose 2	6	6	0.825 (0.22)	
	6 h 15 min	2nd ET-1	6	6	0.608 (0.20)	–25.605 (19.07)
	6 h 30 min	2nd ET-1	6	6	0.490 (0.17)	–39.067 (19.73)
	6 h 45 min	2nd ET-1	6	6	0.457 (0.10)	–43.578 (8.77)
	7 h	2nd ET-1	6	6	0.467 (0.15)	–41.957 (16.44)
	7 h 15 min	2nd ET-1	6	6	0.487 (0.23)	–40.948 (22.98)
	7 h 30 min	2nd ET-1	6	6	0.490 (0.29)	–40.100 (31.88)
S-1039 15.7 µg kg ^{–1} min ^{–1}	1 h 25 min	1st ET-1 pre dose 1	13	12	1.301 (0.42)	
	1 h 30 min	1st ET-1 pre dose 2	13	12	1.232 (0.43)	
	1 h 45 min	1st ET-1	13	12	1.105 (0.39)	–9.580 (12.68)
	2 h	1st ET-1	13	12	1.094 (0.44)	–10.263 (17.16)
	2 h 15 min	1st ET-1	13	12	1.068 (0.50)	–14.168 (18.17)
	2 h 30 min	1st ET-1	13	12	1.044 (0.48)	–16.028 (19.77)
	2 h 45 min	1st ET-1	13	12	0.919 (0.37)	–24.580 (16.92)
	3 h	1st ET-1	13	12	0.944 (0.47)	–23.484 (19.67)
	5 h 45 min	2nd ET-1 pre dose 1	13	11	0.888 (0.21)	
	6 h	2nd ET-1 pre dose 2	13	11	0.878 (0.33)	
	6 h 15 min	2nd ET-1	13	11	0.822 (0.23)	–3.703 (13.59)
	6 h 30 min	2nd ET-1	13	11	0.715 (0.16)	–14.210 (18.09)
	6 h 45 min	2nd ET-1	13	11	0.675 (0.14)	–17.091 (21.76)
	7 h	2nd ET-1	13	11	0.612 (0.16)	–24.908 (22.45)
	7 h 15 min	2nd ET-1	13	11	0.666 (0.22)	–20.802 (21.71)
	7 h 30 min	2nd ET-1	13	11	0.579 (0.20)	–29.535 (21.45)

Subject 117 (S-0139/SB-737004) completed only 15 min of the first ET-1 infusion and then withdrew, therefore subject 117 has been excluded from the summary statistics.

(% change × min) ($P = 0.009$, 95% CI 476, 3064) was seen following the second ET-1 administration. The difference in response to the second ET-1 administration compared with the first was on average –124 (% change × min); this was not statistically significant ($P = 0.88$, 95% CI –1789, 1541).

Significant changes in peripheral haemodynamics were not observed in the delayed administration study. However, by comparison with placebo during the 3-h infusion period, systolic BP was up to 10 mmHg lower, HR was up to 8 bpm higher, cardiac index was up to 0.4 l min^{–1} m^{–2} higher and TPRI was up to 30% lower on S-0139.

A small increase in ET-1 plasma concentration was seen after dosing with S-1039 – from a baseline of 0.8 to 1.2 pg ml^{–1} at the end of infusion, somewhat higher than the change seen on placebo, 0.7 to 0.8 pg ml^{–1}.

Pharmacokinetics

The pharmacokinetic data collected during the delayed administration study showed a mean C_{ss} of 1585 ng ml^{–1} (range 1196–1917). This was somewhat lower than the targeted 2000 ng ml^{–1}, but at this concentration range com-

plete blocking of ET-1-mediated vasoconstriction would have been expected. The S-0139 concentration just before the second ET-1 infusion was on average 17 ng ml^{–1} (range 8–131).

Adverse events

Most AEs were of mild to moderate intensity. During study 1, one subject was withdrawn due to a headache following dosing with placebo. During study 2, the most commonly reported AE was headache, reported in the placebo group and by two subjects at each dose level. Dizziness was reported by two subjects at 6.67 µg kg^{–1} min^{–1} and by one subject at 15 µg kg^{–1} min^{–1}. There did not appear to be a relationship between the dose of S-0139 and AEs.

In the delayed administration study the most frequently reported AEs (rated as possibly related to treatment) were headache and nausea, each reported by five subjects and reported together in four cases. Headache was reported by only one subject receiving placebo and nausea was not reported by any subjects receiving placebo.

Figure 4

Study 3 – delayed administration study: mean ($\pm$ SE) plot of % change from baseline in forearm blood flow (FBF) over time ($\pm$ SE) for S-0139 and placebo following the first and second ET-1 administrations. Following the first ET-1 administration a smaller decrease in FBF (AUEC₀₋₉₀) was seen for S-0139 (–1295) compared with placebo (–189). This difference, on average 1894 (% change $\times$ min), was found to be statistically significant ($P = 0.005$, 95% CI 618, 3170). A similar statistically significant difference in FBF response of 1770 (% change $\times$ min) ($P = 0.009$, 95% CI 476, 3064) was seen following the second ET-1 administration. ET-1 and Placebo (1st ET-1 Admin) (–◆–); ET-1 and S-0139/SB-737004 (1st ET-1 Admin) (–■–); ET-1 and Placebo (2nd ET-1 Admin) (–▲–); ET-1 and S-0139/SB-737004 (2nd ET-1 Admin) (–●–)

Discussion

The results from this study confirm that, in man, S-0139 given systemically blocks ET-1 locally induced vasoconstriction, as demonstrated by the FBF model. Pharmacokinetic data show that antagonism of ET-1-induced effect on FBF occurred at steady-state plasma concentrations of 800–2000 ng ml^{–1}. These data agree well with preclinical efficacy data from the middle cerebral artery occlusion models; in particular, in the cat model positive effects were seen at S-0139 levels of 700–1000 ng ml^{–1} [7].

S-0139 did not appear to have an overt effect on systemic haemodynamics, which is consistent with results from another selective ET_A antagonist, BMS-193884 [13]. TAK-044, a non-selective ET antagonist, reduced total peripheral resistance by approximately 25% [9].

In addition, S-0139 did not have a significant effect on circulating ET-1 levels. Increases in circulating ET-1 levels are a marker for antagonism at the ET_B receptor and hence non-selectivity of the antagonist, although it is noted that systemic levels of ET-1 can vary greatly. In both studies, only small increases in ET-1 were seen during infusion of S-0139, by comparison with the increases of up to >30 pg ml^{–1} that occurred following administration of the non-selective antagonist TAK-044. Although clazosentan is generally believed to be a selective inhibitor for ET_A, its selectivity for ET_A is only 63-fold when compared with ET_{B1} [14]. ET_A receptors are found on vascular smooth muscle cells and mediate vasoconstriction. ET_B receptors are also found on smooth muscle, where they induce smooth muscle contraction (ET_{B2}), but are abun-

dant on the endothelium (ET_{B1}), where their activation results in vasodilation mediated by nitric oxide and prostacyclin. As the ET_B acts as a clearance receptor for ET-1, ET_B inhibition may increase levels of ET-1, potentially resulting in increased unwanted ET_A signalling. In fact, during infusion of clazosentan the serum concentration of ET-1 clearly increased in a dose-dependent manner [15]. In contrast, S-0139 is 251-fold more selective for ET_A compared with ET_{B1} [16] and did not cause any significant changes in ET-1 in the present study. These differences might lead to a narrower clinical safety margin for clazosentan compared with S-0139.

S-0139 was able to antagonise the effects of ET-1 3–4.5 h following the end of drug infusion. The observed mean concentration of S-0139 at the start of the second infusion of ET-1 was 17 ng ml^{–1}. On the basis of the results from the pilot and dose–response study, such plasma concentrations are not expected to inhibit the response to ET-1. This is also borne out by comparing the free concentration (S-0139 is 99.5% protein bound) of 0.1 nM (for a total concentration of 17 ng ml^{–1}) against the K_i for the ET_A receptor of 1 nM. A similar observation for a prolonged effect with S-0139 has been noted in a non-clinical efficacy study. In a rat middle cerebral artery occlusion model, intermittent administration of S-0139 elicited a similar decrease in brain injury volume when compared with continuous infusion of S-0139 (Shionogi, unpublished observation).

At present there is no clear explanation for the observed prolonged action. An antagonist-induced change in the system was observed that persists even after the S-0139 plasma concentrations had fallen to non-

pharmacological levels. Based on observed plasma concentrations and receptor binding kinetics [12], no appreciable receptor occupancy is predicted at this time point. However, prolonged binding of S-0139 to the ET_A receptor because of other, unknown factors cannot be ruled out. Alternatively, internalization of the ET_A receptor–S-0139 complex could be involved, reducing the number of available receptors at the cell surface in a time-dependent manner.

By comparison with the dose–response study, during the delayed administration study, full inhibition of ET-1-induced vasoconstriction was not observed and the degree of vasoconstriction seen in absence of S-0139 was larger. The same batch of ET-1 was used for all studies and testing showed that potency was similar for each study. The reason for the difference in the response between the studies is unclear, but may have been related to a slight difference in methodology in infusing ET-1. In the delayed administration study ET-1 was infused via an indwelling intra-arterial catheter compared with a needle in the first two studies. The catheter was advanced much higher up the brachial artery than a needle would reach, which would lead to a number of branches being perfused with ET-1 that would not normally be within the reach of a needle, thereby exposing more muscle mass in the forearm and possibly producing a larger effect upon FBF. This methodology may therefore require higher concentrations of S-0139 to antagonize the effects fully. However, critically the effect of S-0139 on ET-1-mediated vasoconstriction was comparable whether S-0139 was infused concurrently with ET-1 or ET-1 infused 3–4.5 h after the end of dosing, providing support for a prolonged pharmacodynamic effect of S-0139.

The FBF model has shown that S-0139 is a selective endothelin antagonist in man. Antagonism has been demonstrated at concentrations from 800 to 2000 ng ml⁻¹ and appears to persist longer than would be apparent from the plasma pharmacokinetics. The prolonged duration of action for S-0139 suggests the possibility that the drug could be administered using an intermittent or non-continuous dosing regimen for clinical conditions such as ischaemic stroke.

S-0139 is no longer in clinical development; however, these data would have been helpful to aid its further development and also will be of benefit for future ET antagonists in development, to provide information on the likely effective concentration range.

Competing interests

None to declare.

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