Pharmacokinetics and pharmacodynamic effects of ABT-627, an oral ETA selective endothelin antagonist, in humans

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Aims Endothelins (ETs) may play a role in the pathogenesis of a variety of cardiovascular diseases. The present study was designed to investigate the pharmacokinetic and pharmacodynamic effects of the orally active ETA selective receptor antagonist ABT-627 in healthy humans.

Methods Healthy volunteers were included in two studies with cross-over design. Subjects received single or multiple dose (an 8 day period) administration of oral ABT-627 or matched placebo, in a dose range of 0.2–40 mg. The pharmacokinetics of ABT-627 were described and its effects on systemic haemodynamics under resting conditions and on forearm vasoconstriction in response to ET-1 were assessed.

Results ABT-627 was generally well tolerated in both studies, with transient headache being the most reported adverse event (in 62% *vs* 4% during placebo, $P < 0.05$, for Study 1 and in 42% *vs* 60%, $P = 0.2$, for Study 2). ABT-627 was rapidly absorbed, reaching maximum plasma levels at ≈ 1 h post dose. Single dose ABT-627, at a dose of 20 and 40 mg, inhibited ET-1 induced forearm vasoconstriction at 8 h post dose. Eight days ABT-627 treatment, at a dose level of 5 mg and above, also effectively blocked forearm vasoconstriction to ET-1. ABT-627 caused a significant reduction in peripheral resistance as compared with placebo (16 ± 1 *vs* 19 ± 1 , 18 ± 2 *vs* 23 ± 3 , 15 ± 1 *vs* 17 ± 1 AU at 1, 5, 20 mg in Study 2) with only a mild decrease in blood pressure (79 ± 2 *vs* 84 ± 3 , 80 ± 4 *vs* 90 ± 5 , 75 ± 3 *vs* 79 ± 1 at 1, 5, 20 mg in Study 2). ABT-627 caused a moderate dose-dependent increase in circulating immunoreactive ET levels (a maximal increase of 50% over baseline at the 20 mg dose level).

Conclusions The oral ETA receptor blocker ABT-627 is well tolerated, rapidly absorbed, effectively blocks ET-1 induced vasoconstriction and causes a decrease in total peripheral resistance and mean arterial pressure. Our data suggest that ABT-627 may be a valuable tool in treatment of cardiovascular disease.

Keywords: ABT-627, endothelin antagonist, endothelins, humans

Introduction

Endothelins (ETs) are a family of three isopeptides, ET-1, ET-2 and ET-3, which have been shown to be potent vasoactive agents. ET-1, the most important isoform in the cardiovascular system, is produced mainly by endothelial cells through cleavage of a precursor, big ET-1 or

proendothelin-1, by an endothelin converting enzyme (ECE). The biological actions of ET-1 are mediated by endothelin receptors, of which two subtypes have been characterized and cloned [1, 2]. ETA receptors are expressed predominantly in smooth muscle and mediate vasoconstriction. ETB receptors are present on the luminal surface of endothelial cells mediating relaxation through release of endothelium derived relaxing factors, including nitric oxide (NO) and prostacyclin [3, 4]. ETB receptors have also been shown in the medial smooth muscle of human arteries [5], where they can mediate vasoconstriction [6–8].

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Since its discovery [9], ET-1 has been implicated as a pathogenetic factor in a variety of cardiovascular diseases, such as chronic heart failure, essential and pulmonary hypertension and renal failure, due to its actions both as a very potent, long-lasting vasoconstrictor and as a mitogenic cytokine [10]. This has led to the rapid development of pharmacological agents that interfere with the production or actions of ET-1. Recently, various potent selective and nonselective endothelin receptor antagonists have been developed.

So far, experience with orally active ET receptor blockers in humans has been limited to mixed nonselective ET receptor antagonists [11–13]. However, the ETA receptor is increasingly recognized as the main receptor mediating the vasoconstrictive and proliferative effects of ET-1 in humans, whereas the ETB receptor may mediate beneficial effects. The ETB receptor has been shown to have antiproliferative effects in human cells [14], stimulate regeneration of human proximal renal tubule or endothelial cells [15, 16] and to mediate an inhibitory effect on platelet aggregation [17]. Furthermore, intravenous administration of a selective ETB receptor antagonist in healthy volunteers induced systemic vasoconstriction [18] and we recently demonstrated that ETB antagonism in the human forearm, either alone or on a background of ETA antagonism, caused local vasoconstriction [19]. These observations suggest the presence of an endogenous ETB receptor mediated vasodilator tone. In fact, ETA selective blockade may enhance the vascular activity of the antiatherosclerotic molecule NO, most likely by activation of the ETB receptor [19]. Such data would suggest that ETA selective blockers may have additional benefits over nonselective blockers for some of their potential cardiovascular indications [20].

ABT-627 is a potent endothelin antagonist, which is orally bioavailable with a high selectivity for the ETA receptor (K_i ETA = 0.034 nM; K_i ETB = 63.3 nM) [21]. Pre-clinical studies have demonstrated that ABT-627 inhibits mitogen and vascular biological responses induced by ET-1 challenge. In the current study we report on the haemodynamic actions of this orally active ETA selective receptor antagonist in humans. Two questions were addressed: First, does acute administration of ABT-627 cause systemic vasodilation in healthy volunteers and is there a relationship between the pharmacological and pharmacokinetic effects of the drug? For this purpose, the potential of ABT-627 to block a local challenge with ET-1 in the forearm was performed at maximal plasma levels and repeated 8 h later. In a second protocol we studied the haemodynamic effects, the efficacy of ET receptor blockade, the tolerability and the safety of 1 week exposure to ABT-627 in healthy volunteers. In addition, we examined the effect of ABT-627's ETA selective

antagonism on circulating immunoreactive endothelin (iET).

Methods

Subjects

Healthy male subjects ranging in age from 19 to 65 years participated in two studies that were performed in accordance with the Declaration of Helsinki, with the approval of the University Hospital Utrecht Ethics Committee and with the written, informed consent of each subject. Subjects were within 15% of ideal body weight and did not take any medication in the 2 weeks prior to or during the study. Subjects abstained from nicotine for 6 months, from alcohol and caffeine-containing drinks for 72 h, and from food for at least 8 h before any procedures began.

Drugs and drug administration

The chemical structure of ABT-627 is 2R-(4-methoxyphenyl)-4S-(1,3-benzodioxol-5-yl)-1-(N,N-di(*n*-butyl)-aminocarbonyl-methyl)-pyrrolidine-3R-carboxylic acid, hydrochloride salt; molecular weight = 546 g mol⁻¹. For the present clinical studies in humans, we chose to use doses of ABT-627 ranging from 0.2 to 40 mg. This dose range was based on animal studies of specificity and efficacy, the safety profile of ABT-627 in toxicological studies at much higher doses and initial rising dose clinical trials. Clinical supplies were prepared at Abbott Laboratories as an oral solution of 1 mg ABT-627 ml⁻¹ of solution (50% glycerin, 14% alcohol, quantity satisfied with water). The matched placebo consisted of this solution without ABT-627.

Pharmaceutical grade endothelin-1 (ET-1) was obtained from Clinalfa AG (NovaBiochem) and dissolved in sterile physiological saline (0.9%; Baxter Healthcare Ltd) to the desired final concentration. Endothelin-1 was administered intra-arterially at a dose range of 0.1–5 pmol min⁻¹. For intra-arterial infusion of ET-1, the brachial artery of the nondominant arm was cannulated under local anaesthesia (lignocaine 1%; Astra Pharmaceuticals Ltd) with a 22 gauge radiopaque catheter. Patency was maintained by infusion of 0.9% sterile physiological saline via a Medfusion 2001 syringe pump (Medex, Inc.). The total rate of intra-arterial infusion was maintained constant through all intra-arterial studies at 90 ml h⁻¹.

Clinical assessment

The following assessments were performed during the study: physical examination, vital sign measurements, 12-lead ECG, urinalysis, clinical chemistry screen (liver

Figure 1 Effects of single dose administration of ABT-627 on forearm vasoconstriction to locally infused ET-1 in Study 1. On two separate occasions subjects received one dose of ABT-627 (●) or placebo (□), in three dose groups: 1 mg, $n=6$; 20 mg, $n=12$; 40 mg, $n=6$. At two timepoints, 30 min and 8 h postdose, ET-1 was infused into the brachial artery (1 pmol min^{-1} for 60 min). Endothelin-1 caused slow-onset vasoconstriction after placebo administration. There was no effect of ABT-627 on the 30 min postdose ET-1 responses. Forearm vasoconstriction was attenuated 8 h postdose in the 20 and 40 mg ABT-627 group but not in the 1 mg dose group.

enzymes, electrolytes, creatinine, blood urea, protein), haematology screen (full blood cell count, white blood cell differential count) and self report by the subject. All adverse events were recorded.

Forearm blood flow

All experiments were performed in a quiet room, maintained at a controlled temperature between 22°C and 24.5°C . Subjects rested recumbent throughout forearm blood flow (FBF) measurements with both

forearms resting slightly above the level of the heart. FBF was measured by R-wave triggered venous occlusion plethysmography (Hokanson EC-4 plethysmograph, Hokanson, Inc., Issaquah, Washington, USA) of the cannulated arm as well as of the control arm, using mercury-in-silastic strain gauges applied to the widest part of the forearm [22, 23]. A personal computer (486-DX, Compaq) extended by disposable transducer (Onmeda, The BOC Group) was used for R-wave triggered control of the rapid cuff inflator (Hokanson E-10) and for on-line analysis of FBF and heart rate. Calibration of the

Figure 2 Time profiles of the mean plasma concentrations of ABT-627 following single oral administration at three dose levels (1 mg ●, $n = 6$; 20 mg ■, $n = 12$; 40 mg ◆, $n = 6$).

plethysmograph was done on the day of the measurements using the internal standard of the plethysmography unit. Upperarm cuffs were intermittently inflated to 40 mmHg. Recordings of FBF were made over 2 min periods at 5 min intervals. During measurements of FBF, the hands were excluded from the circulation using a small wrist cuff, inflated to 40 mmHg above systolic blood pressure. Initial baseline FBF measurements were performed at least 30 min after cannulation of the brachial artery.

Systemic haemodynamics

Blood pressure was measured with a mercury sphygmomanometer or automated blood pressure device or, during FBF assessments, using continuous intra-arterial measurements. Cardiac function (stroke volume, cardiac output, and heart rate) was measured with bioimpedance cardiography, using a noninvasive bioimpedance methodology (BoMed NC-COM3, BoMed Medical Manufacturing Ltd, Irvine, California, USA). Comparative research has shown a close correlation with invasively measured cardiac output [24, 25].

Pharmacokinetic and endothelin assays

Venous blood samples were obtained from the arm contralateral to the infusion arm at intervals for assay of serum ABT-627 and plasma immunoreactive endothelin concentrations. ABT-627 concentrations were assayed using a validated high performance liquid chromatography (h.p.l.c.) method with fluorescence detection. The limit

of quantification of the assay, defined as the lowest quantifiable amount of compound at which the loss precision was $< 20\%$ and the accuracy was between $\pm 20\%$, was 0.29 ng ml^{-1} of ABT-627.

For Study 2 only, plasma immunoreactive endothelin was measured by a modified sandwich enzyme immunoassay (EIA) using reagents and kit from R & D Systems (Minneapolis, MN). The sensitivity of the assay is 0.95 pg ml^{-1} immunoreactive endothelin. The assay does not cross-react with ABT-627. Cross-reactivity of the assay with endothelin-1, endothelin-2, and endothelin-3 is 100%, 45%, and 14%, respectively.

Study design

Study 1: Haemodynamics and forearm vasoconstriction to ET-1 after acute dosing of ABT-627

This was an open-label, single-centre study of three selected single oral doses of ABT-627 in healthy adult male subjects. Subjects received 1 mg ($n = 6$), 20 mg ($n = 12$) or 40 mg ($n = 6$) of ABT-627. Studies were repeated with placebo after an interval of at least 14 days. For each period, subjects were admitted the day prior to the assessment. The morning of the day of dosing, after fasting 8 h, blood and urine samples were obtained. Baseline FBF measurements during saline coinfusion were performed for 30 min before dosing. Following dosing with ABT-627 or placebo, a saline baseline measurement over 30 min preceded brachial artery infusion of ET-1 (1 pmol min^{-1}) for 60 min from 30 min to 1 h 30 min post dose. Subjects remained recumbent, were fed a light snack 4 h post dose. At 7 h 30 min post dose the 30 min saline baseline was repeated followed by a 60 min ET-1 infusion.

Measurements were made of forearm blood flow, intra-arterial blood pressure and cardiac function (cardiac output, heart rate and stroke volume) at 5 min intervals during the baseline periods, and during the 60 min ET-1 infusions. Systemic haemodynamics (blood pressure and cardiac function) were measured at regular intervals throughout the study (until 47 h 45 min post dose). Blood samples were obtained at intervals for assay of ABT-627 (see Figure 2). Subjects were discharged two days after dosing following safety assessment.

Study 2: Effects of multiple dose administration of ABT-627 on pharmacokinetics, haemodynamics and forearm vasoconstriction to ET-1

Eight subjects were recruited into each of four dose groups (0.2 mg, 1 mg, 5 mg and 20 mg) of a double-blind, randomized, two-period cross-over, placebo-controlled study with at least 57 days between periods. During period I each subject was randomly assigned to receive one daily dose of either ABT-627 or placebo. During period II

subjects crossed over to receive the alternate treatment. For each period, subjects were admitted to the research unit 1 day prior to dosing (day -1) and dosed daily for 8 days. On day 8, 4 h post dose, 30 min baseline observations were made followed by ET-1 infusion. ET-1 was infused such that at 30 min intervals the dose was increased in the following ascending sequence: 0.1, 0.5, 1 and 5 pmol min⁻¹ for a total 120 min infusion to allow us to test the ET blocking effects of ABT-627 over a wide range of local ET-1 levels.

At 5 min intervals during the 30 min saline infusion and during the 120 min ET-1 infusion measurements were made of forearm blood flow, intra-arterial blood pressure and cardiac function (cardiac output, heart rate and stroke volume). Systemic haemodynamics (blood pressure and cardiac function) were measured at regular intervals on day 8 (from pre dose until 36 h post dose). Blood samples were obtained at multiple timepoints on day 1, 6, 7, 8 and 9 for assay of ABT-627 and iET. Subjects were discharged on day 10.

Data presentation and statistical analysis

Forearm blood flow Plethysmographic data listings were taken from the computer data file, and FBFs calculated for individual venous occlusion cuff inflations. Blood flow in both forearms was obtained from the mean of the last five or six consecutive recordings of each measurement period. To reduce the variability of blood flow data, the ratio of flows in the infused and noninfused arms (M/C-ratio) was calculated for each time point, and expressed as a percentage change from the basal flow rate measurement, using the noninfused arm as a contemporaneous control for the infused arm [26]. Basal flow rate was taken as the average of the last four FBF measurements before dosing (Study 1) or before infusion of ET-1 was begun (Study 1 and 2). Results were analysed using a repeated measures analysis of variance (SigmaStat; Jandel Corp.) [19]. Significance was taken at the 5% level. Data are expressed as means $\pm$ s.e.mean.

Systemic haemodynamics Mean arterial pressure (MAP) was calculated as diastolic blood pressure plus one third of pulse pressure. Data for stroke volume and cardiac output were corrected for body surface area, calculated according to standard nomogram, and described as stroke index (SI) and cardiac index (CI). Total peripheral resistance index (TPRI) was calculated as mean arterial pressure divided by cardiac index and expressed in arbitrary units (AU). For analysis of systemic haemodynamics data obtained over periods of baseline FBF measurements were used, as these were consecutive measurements, acquired under standardized conditions and therefore most reliable. Analysis of variance was used to compare the effects of ABT-627 and placebo at each dose level.

ABT-627 pharmacokinetics Individual pharmacokinetic parameters of ABT-627 were determined with noncompartmental methods. The parameters determined included the maximum observed plasma concentration (C_{max}), time to observed maximum concentration (t_{max}), area under the plasma concentration-time curve (AUC) and terminal elimination half-life.

Results

General

In Study 1, a total of 25 subjects were studied, 24 of whom completed both placebo and ABT-627 treatment, $n=6$ at 1 mg, $n=12$ at 20 mg, and $n=6$ at 40 mg. One subject withdrew from the 20 mg cohort for nonstudy-related reasons and was replaced. A total of 33 subjects were enrolled in Study 2, 29 of whom completed both treatment periods, $n=7$ in each dose group of 0.2 mg, 5.0 mg, and 20 mg; and $n=8$ in the 1.0 mg group. Three subjects withdrew during the placebo treatment period, one due to diarrhoea and two for administrative reasons. One subject withdrew from the 20 mg ABT-627 treatment group due to nonstudy-related reasons.

ABT-627 was generally well tolerated in both studies. There were no serious adverse events in either study, and no clinically significant abnormalities were detected in physical examinations, ECG and clinical laboratory tests. Transient headache was the most reported adverse event. In Study 1, with acute dosing, headache occurred in 4% (1/24) of placebo subjects and 62% (15/24) of ABT-627 subjects, $P<0.05$. However, in Study 2, with chronic dosing, headache occurred in 60% (18/30) of subjects during placebo and 42% (14/33) of subjects during ABT-627 treatment periods, $P=0.2$. There were no statistical differences between ABT-627 and placebo treatment for other adverse events.

Study 1: Acute effects of ABT-627 on pharmacokinetics, haemodynamics and forearm vasoconstriction to ET-1

Forearm vascular responses There were no significant differences in baseline FBF in the infused or control arm prior to dosing in either dose group between the period of active treatment and placebo (see Table 1). Accordingly, baseline M/C-ratios prior to treatment were not significantly different (ABT-627 vs placebo: 1.1 ± 0.1 vs 1.3 ± 0.3 , 1.0 ± 0.2 vs 1.1 ± 0.2 and 1.1 ± 0.2 vs 1.1 ± 0.2 for the 1, 20 and 40 mg dose group, respectively). Oral administration of ABT-627 (1, 20, 40 mg) did not significantly influence basal FBF in the infused or control arm as compared to placebo at 30 min or 8 h post dose (Table 1). At each dose level, for ABT-627 and placebo, there was a tendency towards an increase in absolute values

Table 1 Baseline forearm blood flows in three phases of study 1.

	predose	30 min postdose	8 h postdose	predose	30 min postdose	8 h postdose
<i>ABT-627 1 mg</i>						
<i>Forearm blood flow (ml 100 ml⁻¹ min⁻¹)</i>						
Infused arm	3.4 ± 0.5	4.0 ± 0.5	4.5 ± 0.6	2.3 ± 0.5	2.4 ± 0.7	5.0 ± 1.2
Control arm	3.4 ± 0.7	3.5 ± 0.6	4.8 ± 1.0	2.3 ± 0.5	2.8 ± 0.7	5.7 ± 1.0
<i>ABT-627 20 mg</i>						
<i>Forearm blood flow (ml 100 ml⁻¹ min⁻¹)</i>						
Infused arm	2.2 ± 0.3	2.4 ± 0.3	5.3 ± 0.7	1.8 ± 0.2	2.1 ± 0.3	4.4 ± 0.5
Control arm	2.4 ± 0.3	2.9 ± 0.4	4.8 ± 0.7	2.1 ± 0.4	2.2 ± 0.3	4.8 ± 0.5
<i>ABT-627 40 mg</i>						
<i>Forearm blood flow (ml 100 ml min⁻¹)</i>						
Infused arm	1.9 ± 0.5	1.9 ± 0.5	3.7 ± 0.8	2.2 ± 0.6	2.5 ± 0.7	4.1 ± 0.9
Control arm	2.0 ± 0.3	1.9 ± 0.4	4.1 ± 0.6	1.9 ± 0.2	2.1 ± 0.3	3.7 ± 0.4

Forearm blood flows in infused and control arm during saline coinfusion in three phases relative to dosing of ABT-627 or placebo at three dose-levels: 1 mg ($n=6$), 20 mg ($n=12$), 40 mg ($n=6$) (Study 1): predose; 30 min postdose and 8 h postdose. Data are expressed as mean ± s.e.mean. There were no significant differences in baseline forearm blood flow between ABT-627 or placebo treatment.

of FBF in both the infused and control arm, 8 h post dose, with unaltered M/C-ratios. However, as this tendency occurred both in the ABT-627 as well as in the placebo treated group with no significant differences in flows at 8 h post dose between ABT-627 or placebo dosing, this does not reflect an ABT-627 effect but may rather be due to circadian variation in vascular tone [27] or reflect the vasodilator effects of food intake [28].

ET-1 infusion (1 pmol min⁻¹) during placebo caused significant vasoconstriction at each dose level and in both infusion-blocks ($P<0.01$, mean $28 \pm 6\%$ vasoconstriction at 60 min). No statistically significant differences were observed between ABT-627 as compared with placebo for vasoconstriction in response to ET-1 in any dose group at 30 min post dose ET-1 infusion. However, during the 8-h post dose ET-1 infusion significant vasoconstriction only occurred in the 1 mg ABT-627 treated group ($P<0.01$), with no significant vasoconstrictor response in the 20 mg or 40 mg dose groups ($P=0.48$ and $P=0.96$, respectively). Forearm vasoconstriction to ET-1 was significantly blocked at the 40 mg dose level ($P<0.01$ vs placebo). A trend towards significant differences also occurred at the 20 mg dose level during the 8 h post dose ET-1 infusion but there was no significant effect of 1 mg ABT-627 on the 8-h post dose ET-1 response. [Figure 1].

Systemic haemodynamics For each dose level, mean values for cardiac measurements (stroke volume, cardiac output and heart rate), systolic and diastolic blood pressure and mean arterial pressure prior to dosing were similar for ABT-627 and placebo treatment periods. During the day, in both the ABT-627 as well as the placebo treatment periods, there was a trend towards a decrease in total peripheral resistance index and mean arterial pressure and an increase in cardiac index and heart rate. This may be due to diurnal variation or

the haemodynamic effects of food intake [29]. The decrease in total peripheral resistance and increase in cardiac index tended to be larger after ABT-627 than after placebo dosing at the 40 mg dose level (for TPRI at 30 min post dose: $P<0.05$; for CI at 30 min post dose and at 8 h post dose $P<0.05$). The decrease in mean arterial pressure 8 h post dose was slightly larger after ABT-627 than after placebo in the 20 mg dose group (11 vs 6 mmHg, $P=0.03$). Changes in heart rate were not significantly different following ABT-627 or placebo dosing (Table 2).

ABT-627 pharmacokinetics With the exception of the 1 mg dosing group, plasma concentrations of ABT-627 increased rapidly (median $t_{\max} = 0.5$ h at the 20 mg and 2.0 h at the 40 mg dose level) after single dose oral administration of ABT-627 as a solution formulation, thereafter declining with a terminal half-life of ≈ 24 h. The AUC values were dose proportional. In the 1 mg dosing group, a distributive phase was not apparent and the $t_{\max}$ was longer (median $t_{\max} = 3.0$ h). The pharmacokinetic parameters of ABT-627 for the 3 dosing groups are summarised in Table 3 and the mean ABT-627 plasma concentration-time profiles for the dose groups are shown in Figure 2.

Study 2: Effects of multiple dose administration of ABT-627 on pharmacokinetics, haemodynamics and forearm vasoconstriction to ET-1

Forearm vascular responses Eight days oral administration of ABT-627 at the 0.2, 1 or 5 mg dose level did not significantly alter baseline FBF in either the infused or control arm as compared with placebo (Table 4). Consequently, at these dose levels M/C-ratios were not significantly different following ABT-627 compared

Table 2 Systemic haemodynamic effects of single dose ABT-627 administration (Study 1).

ABT-627/placebo	Predose		30 min postdose		8 h postdose	
	ABT-627	Placebo	ABT-627	Placebo	ABT-627	Placebo
<i>1 mg</i>						
MAP (mmHg)	90 ± 3	89 ± 3	95 ± 4	91 ± 4	83 ± 3	85 ± 6
HR (beats min ⁻¹)	68 ± 4	65 ± 3	70 ± 4	66 ± 3	82 ± 4	76 ± 3
CI (l min ⁻¹ m ⁻²)	3.1 ± 0.2	2.9 ± 0.3	3.2 ± 0.3	3.0 ± 0.3	3.7 ± 0.3	3.7 ± 0.5
TPRI (AU)	30 ± 2.9	31 ± 2.9	31 ± 3.5	31 ± 2.8	23 ± 2.8	24 ± 3.7
<i>20 mg</i>						
MAP (mmHg)	86 ± 3	87 ± 2	89 ± 2	91 ± 2	75 ± 1*	81 ± 2
HR (beats min ⁻¹)	56 ± 2	53 ± 2	59 ± 2	55 ± 2	66 ± 3	63 ± 3
CI (l min ⁻¹ m ⁻²)	3.3 ± 0.3	3.1 ± 0.2	3.4 ± 0.3	3.1 ± 0.1	4.6 ± 0.4	4.3 ± 0.3
TPRI (AU)	29 ± 3.0	29 ± 1.8	29 ± 2.7	30 ± 1.9	18 ± 1.7	20 ± 2.3
<i>40 mg</i>						
MAP (mmHg)	93 ± 4	91 ± 3	96 ± 4	94 ± 3	86 ± 4	85 ± 4
HR (beats min ⁻¹)	58 ± 3	55 ± 3	64 ± 3	61 ± 3	74 ± 3	65 ± 5
CI (l min ⁻¹ m ⁻²)	3.1 ± 0.3	3.1 ± 0.3	3.4 ± 0.3*	3.2 ± 0.3	4.9 ± 0.4*	4.3 ± 0.3
TPRI (AU)	32 ± 3.6	30 ± 2.6	30 ± 3.2*	30 ± 2.8	18 ± 1.9	21 ± 1.9

Systemic haemodynamics after single dose administration of ABT-627 or matched placebo at three dose levels: 1 (*n* = 6), 20 (*n* = 12) and 40 mg (*n* = 6). * *P* < 0.05 for changes from predose, compared to placebo. Data are expressed as mean ± s.e.mean.

with placebo treatment (1.3 ± 0.4 vs 1.3 ± 0.3 ; 0.9 ± 0.1 vs 1.0 ± 0.1 ; 1.0 ± 0.2 vs 0.9 ± 0.1 , for the 0.2; 1; 5 mg groups, respectively). However, oral treatment with 20 mg ABT-627 caused a small but significant elevation of baseline FBF in both the infused as well as in the control arm as compared with the placebo period (see Table 4; *P* = 0.05 and *P* = 0.04, for the infused and control arm, respectively). Baseline M/C-ratios were not significantly different at the 20 mg dose level (1.0 ± 0.1 for ABT-627 vs 1.3 ± 0.2 for placebo treatment; *P* = 0.26).

Brachial artery infusion of ET-1 in incremental doses (0.1, 0.5, 1 and 5 pmol min⁻¹) in all four placebo periods caused a significant slow onset local forearm vasoconstriction (*P* < 0.05 at all dose levels) progressing from a mean vasoconstriction of $20 \pm 4\%$ at 30 min, to $21\% \pm 6\%$ at 60 min, $30 \pm 9\%$ at 90 min to $57 \pm 4\%$ vasoconstriction at 120 min. Forearm vasoconstriction to ET-1 was significantly reduced over the four doses of ET-1 infusion at the 20 mg dose level (*P* < 0.05). There was a trend towards differences over the four doses of ET-1 infusion at the 5 mg ABT-627 dose level. There were no significant effects on ET-1 induced vasoconstriction of ABT-627 at the 0.2 or 1 mg dose level (Figure 3).

To exclude the possibility that lower local concentrations of ET-1 due to increased basal flow following 20 mg ABT-627 treatment might have contributed to the observed reduction of ET-1 induced vasoconstriction, data for all dose groups were also analysed by use of concentration-response curves. This analysis yielded similar results as described above (data not shown). Furthermore, previous studies suggested that percentage response is independent of basal blood flow, except at very high or very low flow rates [26, 30]. This is supported by our present observations in Study 1, showing similar ET-1 induced vasoconstriction following placebo administration at both 30 min and 8 h, despite significant differences in basal flows at these timepoints.

Systemic haemodynamics

Following 8 days of treatment with ABT-627 as compared with placebo there was a trend towards a decrease in mean arterial pressure (*P* < 0.05 at the 5 mg dose level), a decrease in total peripheral resistance index (*P* < 0.05 at 1,

Table 3 Pharmacokinetic parameters (mean ± s.d.) following single dose of ABT-627 (Study 1).

Parameter	ABT-627 dose		
	1 mg	20 mg	40 mg
<i>C</i> _{max} (ng ml ⁻¹)	1.7 ± 1.0	93.5 ± 55.9	166.3 ± 131.5
<i>t</i> _{max} (h)	6.0 ± 10.8	0.6 ± 0.4	1.8 ± 0.6
AUC (ng ml ⁻¹ h)	45 ± 19	801 ± 257	1713 ± 1036
<i>t</i> _{1/2} (h)	26.2 ± 8.3	27.6 ± 6.8	20.1 ± 3.7

Figure 3 Chronic dosing effects of ABT-627 on forearm vasoconstriction in response to brachial artery infusion of incremental doses of ET-1 in Study 2. In two separate periods subjects received one daily dose of ABT-627 (●) or placebo (□) for 8 days (in four dose groups: 0.2 mg, 1 mg, 5 mg, 20 mg; $n=8$ in each group). On day 8, 4.5 h postdose, ET-1 was infused locally in increasing doses (0.1, 0.5, 1 and 5 pmol min^{-1} sequentially, each for 30 min). ET-1 caused significant progressive vasoconstriction over the four doses of ET-1 in the placebo periods. Forearm vasoconstriction to ET-1 was significantly blocked at the 20 mg ABT-627 level and showed a trend towards statistically significant reduction at the 5 mg dose level ABT-627 at 0.2 or 1 mg did not influence ET-1 induced vasoconstriction.

5 and 20 mg) and an increase in cardiac index ($P<0.05$ in the 20 mg dose group) and heart rate ($P<0.05$ at the 5 and 20 mg dose level) (Table 4).

ABT-627 pharmacokinetics Plasma concentrations of ABT-627 increased with increasing dose and were higher after multiple dose administration compared with single dose administration. As expected from the terminal phase half-life (≈ 30 h), steady state was reached within 6 days of dosing. There was little or no significant effect of dose on terminal half-life (Table 5).

ABT-627 dose dependently increased plasma iET levels, with a maximal increase at the 20 mg dose level showing a 50% increase over baseline. Treatment with ABT-627 at low doses, 1 mg or below, caused an increase in iET only after several days of treatment. At the 20 mg dose an elevation was observed after 4 h of initial dosing. There appears to be a correlation between increasing the dose and time that the steady state iET levels are reached (Table 6).

Discussion

The present study is the first to report on the pharmacodynamic and pharmacokinetic effects of an orally available ETA selective endothelin receptor antagonist in humans. Our current data show that oral administration of the ETA receptor blocker ABT-627 is well tolerated and effectively blocks vasoconstriction induced by exogenous ET-1 in healthy humans both after single as well as after multiple dose administration. In addition, ABT-627 causes a mild decrease in mean arterial pressure and total peripheral resistance in these normotensive subjects.

ABT-627 was readily absorbed, reaching maximum mean plasma levels about 1 h post dose. Plasma concentrations were predictably higher after multiple compared with single dose administration. The effective dose range was above 20 mg for single and above 5 mg for multiple ABT-627 dosing. In this dose range 8 days ABT-

Table 4 Effects of multiple dose administration of ABT-627 on forearm blood flow and systemic haemodynamic parameters in Study 2.

	0.2 mg		1 mg		5 mg		20 mg	
	ABT-627	Placebo	ABT-627	Placebo	ABT-627	Placebo	ABT-627	Placebo
<i>Forearm blood flow (ml 100 ml⁻¹ min⁻¹)</i>								
Infused arm	4.7 ± 0.9	3.1 ± 0.8	3.6 ± 0.7	3.3 ± 0.5	3.5 ± 1.0	2.5 ± 0.6	4.8 ± 0.7*	2.9 ± 0.5
Control arm	4.7 ± 0.8	2.9 ± 0.6	4.9 ± 1.0	3.9 ± 0.7	3.3 ± 0.7	2.9 ± 0.5	5.3 ± 0.8*	2.7 ± 0.7
<i>Systemic haemodynamics</i>								
MAP (mmHg)	76 ± 3	76 ± 3	79 ± 2	84 ± 3	80 ± 4*	90 ± 5	75 ± 3	79 ± 1
HR (beats min ⁻¹)	72 ± 3	68 ± 2	70 ± 3	70 ± 2	79 ± 4*	67 ± 2	75 ± 2*	65 ± 4
CI (l min ⁻¹ m ⁻²)	5.4 ± 0.2	5.7 ± 0.2	5.1 ± 0.2	4.7 ± 0.3	4.7 ± 0.4	4.2 ± 0.3	5.5 ± 0.4*	4.9 ± 0.4
TPRI (AU)	14 ± 0.7	14 ± 0.8	16 ± 1.0*	19 ± 1.3	18 ± 1.9*	23 ± 2.5	15 ± 1.2*	17 ± 1.4

Forearm blood flows (FBFs) in infused and control arm and systemic haemodynamics after 8 days oral ABT-627 administration at four dose levels: 0.2 mg (*n* = 7), 1 mg (*n* = 8), 5 mg (*n* = 7), 20 mg (*n* = 7) (Study 2), 4 h postdose, before infusion of ET-1. Data are expressed as mean ± s.e.mean. * *P* < 0.05 *vs* placebo.

627 treatment effectively blocked ET-1 induced vasoconstriction. Following single dose administration ABT-627 also inhibited constriction to ET-1 at 8 h post dose. However, ABT-627 did not influence the vasoconstrictor response to endothelin infusion between 30 and 90 min post dose, approximately the period of peak plasma levels. This is consistent with previous studies demonstrating slow-onset forearm vasodilation in response to local ETA receptor antagonism [19, 22] and is probably due to the quasi-irreversible nature of endothelin binding to its receptor [31].

The response to ET-1 infusion following placebo appears variable. However, there is no statistically significant difference between the response to ET-1 following placebo at 30 min or 8 h post dosing. The apparent increases in response to ET-1 between the 1, 20, 40 mg dose level in Study 1 or between the different dose ranges in Study 2 are also not statistically significant. The apparent differences are most likely due to differences in patient characteristics between groups. For example, the ages in Study 1 varied from 43 ± 6 years at the 1 mg dose level and 37 ± 5 years at the 20 mg dose level to

26 ± 2 years at the 40 mg dose level (mean ± s.d.). Previously, decreased responsiveness to ET-1 with advancing age has been postulated.

There has been some concern about the safety of endothelin receptor blockers, particularly with regard to elevations in hepatic transaminases. ABT-627 was generally well tolerated in the present studies. There were no serious adverse events and no elevations in serum hepatic enzymes were observed. Headache was the most reported adverse event, mostly of mild intensity.

ABT-627 caused significant decreases in peripheral resistance with only mild blood pressure lowering effects. This is probably due to homeostatic mechanisms causing a compensatory increase in heart rate and cardiac index. A similar tendency towards increased pulse rate has been reported in healthy subjects in response to nonselective endothelin receptor antagonists [11, 32]. However, in patients with heart failure and hypertension, conditions which are frequently associated with high plasma endothelin-1 concentrations [33, 34], the nonselective endothelin antagonist bosentan caused significant reduction in blood pressure without any

Table 5 Pharmacokinetic parameters of ABT-627 obtained after single and multiple dose administration of 0.2, 1, 5, and 20 mg once daily doses of ABT-627 (Study 2).

Parameter	Day	0.2 mg	1 mg	5 mg	20 mg
<i>C</i> _{max} (ng ml ⁻¹)	1	0.21, 0.53 #	0.7 ± 0.2	5.4 ± 2.1	36.3 ± 10.0
<i>t</i> _{max} (h)	1	4.12 #	5.0 (1.0–12.0)	1.0 (1.0–6.0)	1.0 (1.0–6.0)
AUC(0,24h) (ng ml ⁻¹ h)	1	0.9, 7.6 #	11.8 ± 3.5	74 ± 25	425 ± 109
<i>C</i> _{max} (ng ml ⁻¹)	8	0.5 ± 0.2	2.2 ± 0.4	10.6 ± 4.2	58.1 ± 19.3
<i>t</i> _{max} (h)	8	4.1 (1.0–12.0)	12.0 (3.0–12.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)
AUC(0,24h)(ng ml ⁻¹ h)	8	8.8 ± 3.7	38.7 ± 5.6	144 ± 32	660 ± 185
<i>t</i> _{1/2} (h)	8	28.9 ##	27.6 ± 9.6	23.6 ± 11.4	30.0 ± 16.5

Data are expressed as mean ± s.d.. # *n* = 2; ## *n* = 1.

Table 6 Trough plasma levels (mean $\pm$ s.e.mean) of ET-1 (pg ml⁻¹) in subjects dosed with ABT-627 (Study 2).

Day	ABT-627				
	0.2 mg	1 mg	5 mg	20 mg	Placebo
1	2.23 $\pm$ 0.65	2.61 $\pm$ 1.19	2.53 $\pm$ 1.08	2.50 $\pm$ 0.55	2.38 $\pm$ 0.83
2	2.21 $\pm$ 0.59	2.47 $\pm$ 0.83	3.10 $\pm$ 1.04	3.50 $\pm$ 1.28	2.51 $\pm$ 0.74
8	2.37 $\pm$ 0.45	3.30 $\pm$ 0.95	3.90 $\pm$ 1.20	3.81 $\pm$ 1.05	2.64 $\pm$ 1.09
9	2.06 $\pm$ 0.45	2.76 $\pm$ 0.70	2.84 $\pm$ 1.23	3.92 $\pm$ 1.40	2.63 $\pm$ 0.78

increase in heart rate [13, 35]. This lack of neurohormonal stimulation in 'pathological' endothelin states could be related to the potentiating effects of endothelin on both the sympathetic and renin-angiotensin system [36–38] in these conditions, and may add to their therapeutic benefit.

Interestingly, ABT-627 administration caused a moderate dose-dependent increase in circulating immunoreactive endothelin levels. These findings are in line with recent observations by others, showing increases in plasma endothelin levels following several highly selective ETA receptor antagonists in dogs [39], micropigs [40] and rats [41, 42]. Previously, ETB receptors have been implicated in the clearance of endogenous endothelins [43, 44]. However, it is unlikely that the observed increases in circulating endothelin levels in these studies are due to antagonist actions at the ETB receptor as drug levels reached are far below the inhibitory constants for the ETB receptor. For example, in the present study following 8 days 20 mg ABT-627 treatment mean plasma levels of ABT-627 4 h post dose were 59.1 nM (32.6 ng ml⁻¹), with 99% being protein bound, leaving 0.6 nM free to bind to the receptor whereas the K_i for the ETB receptor is 63.3 nM. Furthermore, treatment with ETB antagonists such as BQ-788 [18] or non selective endothelin antagonists such as bosentan [11] or TAK-044 [45] resulted in rapid and marked elevations, which were very different from the slow and gradual increase observed in the present study. Possibly, upregulation of ET expression and increased vascular ET-1 production due to inhibition of a negative feedback mechanism may be involved. Alternatively, the increase in plasma ET-1 levels may result from displacement of ET-1 from its receptor site. This finding of elevated circulating ET-1 levels following ABT-627 treatment may be of clinical importance as this may result in activation of the unopposed ETB receptors with subsequent endothelium-dependent vasodilation. This is in keeping with our previous study in which we showed that the local forearm vasodilation in response to brachial artery infusion of a selective ETA receptor antagonist was caused in part by increased generation of nitric oxide [19] and supports a benefit of selective ETA blockers over nonselective blockers.

The observed peripheral vasodilation and blood pressure lowering in response to systemic ETA receptor antagonism extend previous studies reporting local vasodilation following brachial artery infusion of an ETA receptor antagonist or ECE-inhibitor [19, 22] and confirm that endogenous endothelin generation contributes to maintenance of blood pressure in humans. Previous studies on the effects on the nonselective endothelin antagonists bosentan [11] and TAK-044 [32] in healthy subjects showed similar systemic haemodynamic effects. Additionally, nonselective endothelin antagonists could reduce blood pressure in essential hypertensive patients [13], decrease systolic blood pressure and increase coronary diameter in patients with coronary artery disease [46] and lower blood pressure and increase cardiac index in severe chronic heart failure [12, 35]. Several investigators have reported on beneficial effects mediated by the ETB receptor [14–17, 19], suggesting a superiority of selective ETA receptor antagonists over nonselective endothelin blockers. Whether selective ETA blockade has additional therapeutic value in humans cannot be determined from the present study and may vary between disease states. To investigate this further direct comparative studies of the different types of endothelin blockers in various pathophysiological conditions are needed.

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