Pharmacology of endothelin receptor antagonists ABT-627, ABT-546, A-182086 and A-192621: ex vivo and in vivo studies

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ABSTRACT
Endothelins (ETs), 21-amino-acid peptides involved in the pathogenesis of various diseases, bind to ET\textsubscript{A} and ET\textsubscript{B} receptors to initiate their effects. Based on the same core structure, we have developed four small-molecule ET receptor antagonists, ABT-627 (atrasentan), ABT-546, A-182086 and A-192621, which exhibit differences in selectivity for ET\textsubscript{A} and ET\textsubscript{B} receptors. In this report, we compare the efficacy, potency and pharmacokinetic properties of these four antagonists, including potency in inhibiting ET-1- or Sarafotoxin 6c-induced vessel constriction in isolated arteries and efficacy in antagonizing ET-1-, big ET-1- or Sarafotoxin 6c-induced pressor responses in rats.

INTRODUCTION
Endothelin (ET), originally isolated from cultured porcine aortic endothelial cells, is a peptide with 21-amino-acid residues [1]. Three distinct members of the ET family, namely, ET-1, ET-2 and ET-3, have been identified in humans through cloning [2]. Binding of ETs to G-protein-coupled receptors in tissues and cells activates various signalling molecules [3]. Two types of mammalian ET receptors, ET\textsubscript{A} and ET\textsubscript{B}, have been characterized. The ET\textsubscript{A} receptor is selective for ET-1 and ET-2, while ET\textsubscript{B} receptor binds ET-1, ET-2 and ET-3 with equal affinity [4–7].

The ET\textsubscript{A} receptor, which predominates in vascular smooth muscle cells, leads to vasoconstrictive and proliferative responses [8]. The results of ET binding to ET\textsubscript{B} receptor, which is abundant on vascular endothelial cells and is present to a lesser extent on vascular smooth muscle cells in some tissues and species, are less clearly understood. However, the ET\textsubscript{B} receptor on the vascular endothelium can mediate vasodilation through the release of nitric oxide [8], and several lines of evidence support the concept that the ET\textsubscript{A} receptor is the primary receptor subtype responsible for clearing ET from the circulation [9].

A major advance was made in the ET field with the development of endothelin receptor antagonists [10]. BQ-123 and FR139317 [11,12], two peptidic ET\textsubscript{A}-selective antagonists, are important tools in the investigation of ET-mediated pathophysiology. Following the peptidic compounds, a number of nonpeptide antagonists with improved pharmacokinetics, such as Ro 47-0203 [13], SB 217242 [14], ABT-627 (atrasentan) [15], etc., were developed. Some of these antagonists are being investigated in human clinical trials [16].

Recent evidence suggests that ET\textsubscript{A} receptor may play a more important pathological role than ET\textsubscript{B} receptor [8]. However, some tissues express ET\textsubscript{B} receptor predominantly, which seems to suggest that ET\textsubscript{B} receptor may also play a pathophysiological role [17]. Thus, ET\textsubscript{A}-selective or ET\textsubscript{B}-selective antagonists could potentially have their unique utilities. Based on the same chemical

Key words: A-182086, A-192621, ABT-546, ABT-627, atrasentan, endothelin, endothelin receptor, endothelin receptor antagonist.

Abbreviations: ET, endothelin; S6c, sarafotoxin 6c; i.v., intravenous; AUC, area under the curve; MAP, mean arterial blood pressure.
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structural core, we have developed four nonpeptide ET receptor antagonists, ABT-627, ABT-546, A-182086 and A-192621, which exhibit differences in selectivity for ET_A and ET_B receptors. The purpose of this report is to compare the potency and pharmacokinetic profiles of these four antagonists utilizing in vivo and ex vivo assay systems.

MATERIALS AND METHODS

ET-1, big ET-1 and sarafotoxin 6c (S6c) were purchased from American Peptide Co. (Santa Clara, CA, U.S.A.). All other reagents were of analytical grade.

Animals

Male Sprague-Dawley rats were purchased from Charles River (Kingston, NY, U.S.A.). New Zealand white rabbits, male, 2.0–2.5 kg, were purchased from Covance (Kalamazoo, MI, U.S.A.). Male and female dogs used in pharmacokinetic studies were purchased from Marshall Research Animals (North Rose, NY, U.S.A.). Female cynomolgus monkeys were obtained from the Abbott Drug Analysis Colony. All protocols utilizing live animals were approved by Abbott Laboratories’ Institutional Animal Care and Use Committee and were conducted in AAALAC (American Association for Accreditation of Laboratory Animal Care) accredited facilities.

Vessel contraction

Antagonism of ET-1- or S6c-induced vasoconstriction by test agents was evaluated using isolated rat aortic rings (mediated by ET_A) or rabbit pulmonary artery rings (mediated by ET_B) [18]. The experimental details and the analysis of data by Schild analysis to calculate pA2 values have been described previously [19].

Pharmacokinetics

The pharmacokinetic behaviour of these antagonists was evaluated in male Sprague–Dawley rats (250–300 g), male or female beagle dogs, and female cynomolgus monkeys as described previously [19].

In vivo pseudoefficacy

The in vivo efficacy of these antagonists was evaluated in male Sprague-Dawley rats (250–350 g) as described previously [19].

Statistics

Statistical analysis of data was performed using StatView II software (Abacus Concepts, Berkely, CA, U.S.A.). Group comparisons were determined by an ANOVA followed by the Fisher’s protected least significant difference test. Unless noted, values are expressed as mean ± S.E.M. and n represents the number of animals or separate experiments in each group. Difference test of \( P < 0.05 \) was considered significant.

RESULTS

Figure 1 shows that A-182086 produced concentration-dependent, parallel rightward shifts in the S6c (an ET_B receptor-selective receptor agonist) concentration–response curve in isolated endothelium-denuded rings of rabbit pulmonary artery (mediated by the vasoconstrictor-type ET_B receptor). The EC_{50} of S6c-induced vasoconstriction was 1.57 nM (n = 6). Schild analysis of the concentration–response curves in the presence of various A-182086 concentrations yielded a pA_2 of 8.00 ± 0.23 (n = 6). The slope of the regression lines was 1.17 ± 0.27 and r = 0.76. The S6c maximal contraction in the tissue was not significantly affected by A-182086. A-182086 (up to concentrations of 10 \( \mu \)M) was devoid of agonist activity.

Antagonism of ET_A receptor by A-182086 was evaluated in endothelium-intact rat aortic rings in which ET-1-induced vasoconstriction is mediated principally by ET_A receptor (results not shown). The EC_{50} of ET-1-induced vasoconstriction was 1.10 nM. A-182086 shifted the ET-1 concentration–response relationship to the right in a concentration-dependent manner, and Schild analysis yielded a pA_2 of 8.53 ± 0.26 (n = 6), with a slope of 0.87 ± 0.14 and r = 0.76. A-182086 alone also did not exhibit any intrinsic agonist activity on baseline contractile tone of endothelium-intact rat aortic rings. Therefore, A-182086 exhibits similar potency for both ET_A and ET_B receptors in this assay system.

The studies were repeated for A-192621. The pA_2 values of A-192621 were 5.21 ± 0.21 (n = 6) and 8.41 ± 0.33 (n = 5) for ET_A and ET_B receptors respectively.
Table 1  Summary of results from *ex vivo* and *in vivo* studies

* A-192621 alone caused elevation of systemic arterial blood pressure; ND, not determined; C\_max, maximal concentration. † All values for ABT-627 and ABT-546 have been reported previously [15,18].

<table>
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<th>Study</th>
<th>Parameters</th>
<th>Types</th>
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<th>ABT-546†</th>
<th>A-182086</th>
<th>A-192621</th>
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Figure 2  Plasma concentrations of A-182086 after a 5 (i.v.) or 10 (oral) mg/kg dose in the rat (n = 4)

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ively. Results for ABT-627 and ABT-546 have been reported previously [15,18]. The average pA₂ values of these four compounds are summarized in Table 1. These data indicate that ABT-627 and ABT-546 are ET\_ₐ-selective, A-192621 is ET\_ᵦ-selective, while A-182086 is a non-selective antagonist.

The pharmacokinetic profile of A-182086 was examined in rat, dog and monkey. In male Sprague-Dawley rats, the pharmacokinetic behaviour of A-182086 following a 5 mg/kg intravenous (i.v.) dose was characterized by an apparent plasma elimination half life of 5.0 h. A-182086 was absorbed from the 10 mg/kg oral dose, with peak plasma concentrations recorded 0.5 h after oral (solution) administration. Peak plasma concentrations averaged 2.36 µg/ml, declining with an apparent elimination half life of 8.1 h. The bioavailability of A-182086 was estimated to be 54% in the rat (Figure 2). Similar studies were performed in the beagle dog and in the cynomolgus monkey, and the results are summarized in Table 1.

The pharmacokinetic studies were repeated for A-192621 in rat, dog and monkey. Results for ABT-627 and ABT-546 have been reported previously [15,18]. The data from these studies are summarized in Table 1. These data suggest that these four antagonists exhibit good bioavailability in rat, dog and monkey.

Measurement of big ET-1- and S6c-induced changes in mean systemic arterial blood pressure in conscious, normotensive rats was used to evaluate the *in vivo* oral efficacy of A-182086 against ET\_ₐ and ET\_ᵦ receptors respectively.

In Figure 3, A-182086 (1–100 mg/kg) or vehicle was administered orally by gavage. S6c (0.3 nmol/kg, i.v.) was administered as a bolus 1 h after dosing. The pressor response was quantified by calculation of the area under the curve (AUC\_MAP) of mean arterial blood pressure.
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Figure 3 Antagonism of S6c-induced (0.3 nmol/kg, i.v. bolus) dilatory and/or pressor responses by A-182086 in conscious rats

(A) Blood pressure responses to exogenous S6c were determined at 1 h after dosing. (B) and (C) The dilatory and pressor responses were quantified by calculation of the AUCMAP measured over time and where MAP was expressed as the percent change from baseline pressure (i.e. average pressure measured over 30 min prior to the S6c challenge). *P < 0.05. p.o., per os (oral).

A-182086 (1–100 mg/kg, administered orally by gavage) also exhibited a dose-dependent inhibition of the peak pressor response to big ET-1 (0.5 nmol/kg, i.v.), and statistically significant inhibition was achieved at doses of 10–100 mg/kg (results not shown). The ED50 is determined as 10 mg/kg.

The in vivo efficacy studies were repeated for A-192621 in the rat with ET-1 for the assessment of its potency against ET\(_A\), and with S6c for ET\(_B\). As predicted, A-192621 inhibited the ET-1-induced dilatory response (mediated by ET\(_B\)), but failed to inhibit the ET-1-induced pressor response (mediated by ET\(_A\)); A-192621 was potent in blocking both the dilatory and pressor responses induced by S6c (mediated by ET\(_B\)) with an ED50 value of 30 mg/kg. One interesting observation was that A-192621 alone, administered orally either by gavage or in food, caused elevation of arterial blood pressure (Figure 4). The results for ABT-627 and ABT-
DISCUSSION

We have reported the development and characterization of A-127722 (ABT-627 or atrasentan) and A-216546 (ABT-546), two novel, nonpeptide ET antagonists that are highly selective for ET$_A$, previously [15,19]. Based on the same core structure, we have developed A-192621 [20], an ET$_B$-selective antagonist, and A-182086 [21], a non-selective antagonist. Data clearly demonstrate that these four compounds exhibit distinct characteristics in term of their selectivity towards the two subtypes of ET receptors. ABT-627 and ABT-546 are selective for ET$_A$, while A-192621 is selective for ET$_B$, and A-182086 is a ‘balanced’ ET$_A$/ ET$_B$ antagonist.

To determine the efficacy of these compounds, we examined the ability of the antagonists to inhibit agonist-induced constriction of vascular tissue utilizing isolated rat aorta and rabbit pulmonary artery. It is known that, in the rat aorta, ET$_A$ is the predominant mediator [18], while in the rabbit pulmonary artery, ET$_B$ mediates agonist-induced responses [22]. All four compounds produce a parallel and rightward shift of the agonist (either ET-1 or S6c) concentration–response curve without affecting maximal force, and appear to act as fully competitive receptor antagonists. The potency and selectivity of these four compounds from this study is consistent with that observed in the receptor binding study.

The pharmacokinetic studies indicate that all four compounds are orally available in the rat, dog and monkey. In addition, in the conscious rat, both ABT-627 and ABT-546 exhibit a dose-related inhibition of the blood pressure response to exogenous ET-1 with ED$_{50}$ values of 1 and 10 mg/kg respectively. In the same animal model, A-182086 inhibits the pressor response induced by big-ET-1 (mediated by ET$_A$) with an ED$_{50}$ value of 10 mg/kg, and both dilatory and pressor responses induced by S6c (mediated by ET$_B$) with ED$_{50}$ values of $\leq$ 3 and 10 mg/kg respectively. As predicted by its selectivity toward the ET$_B$ receptor, A-192621 inhibits both dilatory and pressor responses induced by S6c mediated by ET$_B$ with an ED$_{50}$ value of 30 mg/kg, and failed to inhibit the ET-1-induced pressor response mediated by ET$_A$. A-192621 alone caused elevation of arterial blood pressure and an elevation in the plasma ET-1 level [23], suggesting that the ET$_B$ receptor is likely to be the clearance receptor for ET-1, and is involved in maintaining arterial blood pressure in the conscious normotensive rat.

In summary, this paper show that four compounds derived from the same core structure are highly potent, orally available ET receptor antagonists with different selectivity toward the two ET receptor subtypes.

REFERENCES

ET receptor antagonists sharing same core structure


