Pharmacological Profile of ZD1611, a Novel, Orally Active Endothelin ET<sub>A</sub> Receptor Antagonist

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ABSTRACT

The endothelins (ETs), potent vasoconstrictor peptides, have been implicated in the pathogenesis of various cardiovascular disorders. In the present study, we describe the novel, potent, orally active, selective ET<sub>A</sub> receptor antagonist ZD1611 [3-(4-[3-(3-methoxy-5-methylpyrazin-2-ylsulfamoyl)-2-pyridyl]phenyl)-2,2-dimethylpropanoic acid]. ZD1611 competitively inhibited <sup>125</sup>I-labeled ET-1 binding at human cloned ET<sub>A</sub> and ET<sub>B</sub> receptors with pIC<sub>50</sub> values of 8.6 ± 0.1 and 5.6 ± 0.1, respectively, showing 1000-fold selectivity for the ET<sub>A</sub> receptor. ZD1611 caused a parallel rightward shift of the concentration response curve to ET-1 in the rat isolated aorta yielding a concentration of antagonist that caused a 2-fold rightward shift of the ET-1-response curve (pA<sub>2</sub>) of 7.5 ± 0.3. When administered i.v. to anesthetized rats and dogs, ZD1611 caused dose-related rightward shifts of partial dose-response curves to the precursor of ET-1, big ET-1. Threshold doses for significant antagonist activity were determined as 0.1 mg/kg and 0.3 mg/kg in the rat and dog, respectively. Importantly, ZD1611 was able to reverse an established big ET-1-induced pressor response in pithed rats in the presence of continuous big ET-1 infusion. Failure of ZD1611 to inhibit the BQ3020 (ET<sub>B</sub>-selective)-induced depressor response in pithed rats indicated a lack of activity at the endothelial ET<sub>B</sub> receptor. ZD1611 was orally active in the rat at 0.3 mg/kg and had a duration of action of more than 7 h, and, in the dog, a dose of 0.6 mg/kg p.o. was active for at least 6 h. In conclusion, these data demonstrate that ZD1611 is a potent and orally active, selective ET<sub>A</sub> receptor antagonist with a long duration of action which may be of therapeutic use.

Since its discovery in 1988, endothelin (ET) 1 remains one of the most potent vasoconstrictors yet described (Yanagisawa et al., 1988). Originally isolated from endothelial cells (Yanagisawa et al., 1988), ET-1 belongs to a family of three isopeptides termed ET-1, ET-2, and ET-3 (Inoue et al., 1989). ET-1 is the predominant isof orm in the vasculature and elicits a variety of actions including constriction, dilatation (via the release of nitric oxide or prostacyclin), and cell proliferation (Ohashi and Douglas, 1993; see Masaki, 1995). The ETs mediate their effects via two known receptor subtypes, the ET<sub>A</sub> and the ET<sub>B</sub> receptor. Both receptor subtypes have been sequenced and cloned in animals and humans and belong to the seven-transmembrane, G protein-coupled receptor superfamily (Arai et al., 1990; Sakurai et al., 1992). The receptors may be distinguished pharmacologically, based on the rank order of potency of the ET isoforms, at the ET<sub>A</sub> receptor ET-1 > ET-2 > ET-3; and at the ET<sub>B</sub> receptor, all three isopeptides are equipotent (Sakurai et al., 1992).

Within the vasculature, ET<sub>A</sub> receptors have been localized to smooth muscle cells where they mediate the constrictor and mitogenic actions of ET (Sakurai et al., 1992; Ohlstein and Douglas, 1993). Constrictor ET<sub>B</sub> receptors have also been identified on vascular smooth muscle of a number of animal species (Ihara et al., 1991; Moreland et al., 1992). However, whereas mRNA for ET<sub>B</sub> receptors has been identified in human vascular smooth muscle (Davenport et al., 1995), the functional relevance of this receptor remains unclear, and the ET<sub>A</sub> receptor appears to predominate (Godfraind, 1993; Maguire and Davenport, 1995). ET<sub>B</sub> receptors are also located on endothelial cells where they mediate vasodilatation, via the release of nitric oxide and/or prostacyclin (Warner et al., 1989).

There is now substantial evidence supporting a role for ET in the pathogenesis of various acute and chronic cardiovascular disorders. Raised ET plasma levels have been described in patients with atherosclerosis (Lerman et al., 1991), pulmonary hypertension (Stewart et al., 1991), chronic heart failure (Love and McMurray, 1997), acute and chronic renal failure (Tomita et al., 1989; Stockenhuber et al., 1992), and...
after myocardial infarction (Miyauchi et al., 1989) and subarachnoid hemorrhage (Suzuki et al., 1992). In addition, ET receptor antagonists have beneficial effects in various experimental models of these conditions (Miyauchi et al., 1993; Patel et al., 1996).

If ET receptor antagonists are to be of therapeutic use in the treatment of chronic disease, the development of orally active compounds would be advantageous. Ideally, such antagonists should block the constrictor actions of ET while preserving the dilator effects; i.e., be selective for the ETA receptor. In addition, antagonist selectivity for the ETA receptor is important given that the ETₐ receptor has been implicated in the clearance of plasma ET-1 (Gasic et al., 1992; Fukuroda et al., 1994). Here, we describe the pharmacological profile of ZD1611 [3-[4-[3-(3-methoxy-5-methylpyrazin-2-yl)-2-sulfamoyl]-2-pyridyl]phenyl]-2,2-dimethylpropanoic acid] (Fig. 1), a novel, orally active, selective ETA receptor antagonist. By using established in vitro and in vivo methods, we have evaluated both the potency and the duration of action of ZD1611 and demonstrated a lack of activity at the ETₐ receptor.

Materials and Methods

Preparation of Cell Membranes. The cDNA for human ETₐ and ETₐ receptors were obtained by polymerase chain reaction of a human kidney cDNA library and a human placental cDNA template, respectively. The cDNAs were subcloned and stably expressed in human kidney cDNA library and a human placental cDNA template, and ETB receptors were obtained by polymerase chain reaction of a

Preparation of Cell Membranes. The cDNA for human ETₐ and ETₐ receptors were obtained by polymerase chain reaction of a human kidney cDNA library and a human placental cDNA template, respectively. The cDNAs were subcloned and stably expressed in human erythroleukemic (MEL)-C88 cells according to previously described methods (Needham et al., 1992). MEL-C88 cells identified as expressing cloned human ETₐ or ETₐ receptors were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 2 mg/ml G418, 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM glutamine.

For radioligand-binding experiments, cells were harvested, washed in PBS, and resuspended in homogenization buffer (Tris-HCl, 50 mM; sucrose, 0.18 M; soybean trypsin inhibitor, 5 μg/ml; bacitracin, 100 μg/ml; benzamidine, 1 mM; phenanthroline, 1 mM, pH 7.5). Cell membranes were then prepared by homogenizing the cell suspension with a BioNeb (Indiana University, Bloomington, IN) nebulizer. The homogenate was clarified by centrifugation (1500 g, 15 min at 4°C), and membranes were sedimented after centrifugation of the supernatant (40,000 g for 30 min at 4°C). The resultant pellet was resuspended in homogenization buffer and frozen until used.

Radioligand Binding in Cell Membranes. MEL-C88 cell membranes containing either cloned human ETₐ or ETₐ receptors (1.5 μg of protein per assay well) were incubated with 30 pM ¹²⁵I-labeled ET-1 in the presence of increasing concentrations of ZD1611 (100 pM to 10 μM), in a final incubation volume of 225 μl. Samples were incubated for 180 min at 30°C followed by filtration through Whatman (Maidstone, Kent, UK) GF/B filters with a Brandel (Bethesda, MD) cell harvester. ¹²⁵I-labeled ET-1 binding was quantified by gamma counting. Nonspecific binding was defined by ¹²⁵I-labeled ET-1 binding in the presence of a 3000-fold excess of unlabeled ET-1.

In Vitro Potency in Isolated Rat Aorta. Male Sprague-Dawley rats (300–350 g) were sacrificed by decapitation and exsanguination. The aorta was quickly removed and placed in oxygenated (95% O₂/5% CO₂) physiological salt solution (PSS) composed of 119 mM NaCl, 4.7 mM KCl, 1.6 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 22.6 mM NaHCO₃, 5 mM dextrose, and 0.03 mM EDTA maintained at pH 7.4. The aortae were stripped of adherent fat and connective tissue. Subsequently, ring segments (2-mm long, 2–3 mm i.d.) were cut and denuded of endothelium by gentle abrasion of the intimal surface.

Isometric tension studies were carried out as described previously (Blaustein and Tulenko, 1989). Briefly, each aortic ring was mounted on two stainless steel pins (130-μm radius) and submerged in a 5-ml organ chamber containing PSS gassed continuously with 95% O₂/5% CO₂ and maintained at 37°C. The PSS was supplemented with 1 μM propranolol to prevent potential activation of smooth muscle β adrenoceptors by ET-induced norepinephrine release from intramural neurons (Takimoto et al., 1993).

Vascular rings were placed under their predetermined optimal passive load (approximately 3 g) and equilibrated for 30 min. Isometric tension measurements were made with linear tension displacement transducers (Grass FT03) and recorded on a Grass model 7D polygraph.

After the equilibration period, tissues were exposed to 80 mM KCl to assess viability. During the plateau phase of this contraction, denudation of vascular endothelium was confirmed by the lack of relaxation to 1 μM acetylcholine. The tissues were then washed until the tension returned to baseline levels and equilibrated for another 60 min. Cumulative concentration-response curves (CRCs) to ET-1 were then constructed in the absence or presence of increasing concentrations of ZD1611. ZD1611 was added 60 min before the construction of the CRC to ET-1. One CRC to ET-1 was constructed per preparation and paired segments of rat aorta were studied for comparison. Each experiment was terminated by the addition of 30 mM BaCl₂, and responses to ET-1 are expressed as a percentage of this reference contraction. Changes in isometric tension development, determined from developed wall stress (g/cm²), were calculated and analyzed with Bioreport software (Modular Instruments, Malvern, PA).

The concentration of ET-1 required for half the maximal contraction (EC₅₀) was determined from log-logit plots of individual CRCs and expressed as the negative log of the molar concentration (pD₂). The concentration of antagonist that caused a 2-fold rightward shift in the ET-1-response curve (pA₂) was used as an index of potency for a competitive antagonist. Schild plots were constructed and pA₂ values were calculated by linear regression analysis, if the slope did not differ from unity (Arulnachalam and Schild, 1959). Data are presented as the mean ± S.E.

In Vivo Potency of i.v. ZD1611 in Pithed Rats. Male Alderley Park rats (280–330 g) were anesthetized with 2% halothane and artificially resired through a tracheal cannula. Rats were pithed by passing a needle (2 mm diameter) through the orbit, the foramen magnum, and down the spinal cord. The left femoral vein and right carotid artery were isolated, and heparin-filled catheters were implanted for the administration of compounds and the measurement of mean arterial pressure (MAP), respectively. Body temperature, measured rectally, was maintained at 38°C via a heated pad.

MAP and heart rate (derived from pulse pressure) were allowed to stabilize over a 10-min period, and a baseline reading was taken. Rats with an initial baseline MAP of <55 mm Hg or >70 mm Hg were excluded from the study.

The precursor of ET-1, big ET-1, was used for in vivo analysis of the effects of ZD1611. Exogenously administered big ET-1 is converted to the biologically active peptide ET-1 in vivo (Hemsen et al., 1991) via a phosphoramidon-sensitive ET-converting enzyme (Fukuroda et al., 1990). In the present study, the use of big ET-1 in vivo was preferred because this compound fails to elicit the initial depres-

Fig. 1. Structure of ZD1611 [3-[4-[3-(3-methoxy-5-methylpyrazin-2-yl sulfamoyl)-2-pyridyl]phenyl]-2,2-dimethylpropanoic acid].
sor response associated with i.v. administered ET-1 (Yanagisawa et al., 1988) and yielded a greater maximum response than that to ET-1 itself.

A partial cumulative dose-response curve to i.v. big ET-1 (starting at 0.3 nmol/kg) was constructed until pressor responses >30 mm Hg were achieved. After a 55-min recovery period, ZD1611 (0.03–0.3 mg/kg) or vehicle was administered, and the big ET-1-response curve was repeated 5 min later. The activity of ZD1611 was calculated as a ratio of the dose of big ET-1 required to give a 30-mm Hg rise in MAP in the absence and then the presence of the compound.

Reversal of an Established Big ET-1 Pressor Response by i.v. ZD1611. To study the ability of ZD1611 to reverse big ET-1-induced pressor responses, big ET-1 was infused i.v. in pithed rats (as previously described), at 0.065 nmol/kg/min. Infusion was continued until a sustained increase in MAP of approximately 100 mm Hg was obtained. ZD1611 (0.03–1 mg/kg) or vehicle was then administered i.v. in a cumulative manner, in the presence of the big ET-1 infusion.

Activity of i.v. ZD1611 against BQ3020-Induced Depressor Responses. The selectivity of ZD1611 for the ET₃ₐ-mediated pressor response over the ET₃ₐ-mediated depressor response was investigated in pithed rats. Rats were pithed as previously described. In addition to left femoral vein and right carotid artery cannulation for compound administration and blood pressure/heart rate measurements, respectively, the right jugular vein was cannulated for noradrenaline infusion. Noradrenaline (3 µg/ml) was infused at an initial infusion rate of 30 µl/min, which was increased until MAP was stabilized at 140 to 160 mm Hg. Two partial dose-response curves to the selective ET₃ receptor agonist BQ3020, separated by a 30-min recovery period, were constructed in the presence of the noradrenaline-induced pressor response. ZD1611 (0.0 mg/kg) or vehicle was administered i.v. 5 min before the second response curve.

In Vivo Potency of i.v. ZD1611 in Anesthetized Dogs. To investigate potency in more than one species, ZD1611 was also administered to dogs. Female beagle dogs (9–14 kg) were fasted overnight and premedosed with acetylpromazine (ACP injection, 2 mg/ml) at a dose of 1 mg/dog administered s.c. The animals were subsequently anesthetized with i.v. sodium pentobarbitone (Sagatal, 60 mg/ml) at a dose of 45 mg/kg and anesthesia maintained with a constant infusion of 6 mg/kg/h. Animals were intubated using a 9-mm tracheal tube and artificially respired. The right jugular vein was cannulated with a dual lumen catheter for the infusion of anesthetic and administration of compounds, and the femoral artery for blood pressure measurements. Atenolol (3 mg/kg i.v.) was administered 10 min before vagotomization. After a 30-min stabilization period, a partial cumulative dose-response curve to i.v. big ET-1, with doses that elicited a pressor response >20 mm Hg (0.1–1 nmol/kg), was constructed. Following a 2-h recovery period, ZD1611 (0.1; 0.3 mg/kg) or vehicle was administered, and the big ET-1-response curve was repeated 20 min later. As before, the activity of ZD1611 was calculated as a ratio of the dose of big ET-1 required to give a 20-mm Hg rise in MAP in the absence and then the presence of the compound.

In Vivo Potency of p.o. Administered ZD1611 in Conscious Rats. Male Alderley Park rats (280–330 g) were anesthetized with i.v. Saffan (alfaxalone, 0.9%, w/v; alfadolone, 0.3%, w/v; 1:2.5 distilled water) and fitted with in-dwelling cannulas in the carotid artery and jugular vein. The catheters were externalized at the back of the neck and filled with heparin (5000 U/ml). During the recovery period, rats were housed individually with free access to food and water. Subsequently, the rats were fasted overnight with unlimited water available.

On the day of the experiment, the rats were placed in perspex housing and restrained. The arterial catheter was drained, refilled with heparinized saline (50 U/ml), and connected to a pressure transducer for measurement of MAP. Heart rate was derived from pulse pressure. After a 10-min stabilization period, a partial cumulative dose-response curve to i.v. big ET-1 was constructed until an increase in MAP of 30 mm Hg was achieved. The animals were then returned to their cages and allowed to recover for 2 h. ZD1611 (0.1–0.9 mg/kg p.o.) or vehicle was then administered p.o. during the recovery period, and the big ET-1 dose-response curve was repeated at time intervals of 0.5, 2, and 4 h after dosing. In separate studies, a dose of 1.5 mg/kg p.o. was administered and the big ET-1 response was measured at 0.5, 7-, and 10-h time intervals. As before, the activity of ZD1611 was calculated as a ratio of the dose of big ET-1 required to give a 30-mm Hg rise in MAP in the absence and then the presence of the compound.

In Vivo Potency of p.o. Administered ZD1611 in Dogs. To investigate the oral activity of ZD1611 in dogs, it was necessary to administer ZD1611 to conscious dogs and then assess its activity under anesthesia. Female Alderley Park beagles (8–11 kg) were dosed p.o. with a 10-ml gelatin capsule containing either 0.6 mg/kg ZD1611 in solution or vehicle. After a rest period of 60 min, the dogs were anesthetized and prepared as described above. Partial dose-response curves to big ET-1 were constructed 3 and 6 h after oral dosing. Activity was assessed by comparing the big ET-1 ED₅₀ values between drug- and vehicle-treated groups.

Materials. Big ET-1, ET-1, and BQ3020 were obtained from Cambridge Research Biochemicals (Cheshire, UK). Acetylcholine, atenolol, bacitracin, benzaminidane, BSA, noradrenaline, phenanthrolone, and propranolol were purchased from Sigma Chemical Co. (Poole, UK or St. Louis, MO). Heparin was obtained from C.P. Pharmaceuticauls (Wrexham, UK), and penicillin and streptomycin were purchased from Life Technologies (Paisley, UK). ¹²⁵I-labeled ET-1 was purchased from Amersham International plc (Amer sham Pharma Biotech, UK). Saffan was obtained from Pitman-Moore (Uxbridge, Middlesex, UK) Ltd. and Sagatal obtained from Rhone Merieux. Acetylpromazine was obtained from C-Vet (Leyland, Lancashire, UK). Stock solutions (100 µM) of ET-1 were made by dissolving in distilled H₂O with 0.1% BSA (fraction V) and stored in aliquots at 4°C for <10 days. Working solutions were prepared daily as needed. ZD1611 was dissolved in dimethyl sulfoxide to give 100 µM stock solutions on the day required.

Statistical Analyses. In all cases n equals the number of individual animals studied. pIC₅₀ and pD₂ values (negative log of the IC₅₀ and EC₅₀, respectively) are given as arithmetic mean with S.E.M. Significant difference between the Schild regression slope and unity was tested using the Student’s one-sample t test. Antagonist activity of ZD1611 in vivo was calculated as a ratio of the dose of big ET-1 required to give a 20- or 30-mm Hg rise in MAP in the absence and presence of the compound; ED₅₀ and ED₃₀, respectively. Mean dose ratios are expressed as geometric mean with 95% CIs. Differences between dose ratios were tested by using Students’ t test for unpaired data. A p value < .05 was considered to be significant.

Results

Inhibition of ¹²⁵I-labeled ET-1 Binding by ZD1611 in Human Cloned ET Receptors. In competition-binding experiments with human cloned ET₃ₐ receptors, ZD1611 competed with ¹²⁵I-labeled ET-1 in a monophasic manner with a pIC₅₀ value of 8.6 ± 0.1 (n = 5). ZD1611 was 1000-fold less potent at the human ET₃ₐ receptor with a pIC₅₀ value of 5.6 ± 0.1 (n = 3) (Fig. 2). Slopes were not significantly different from unity.

Selectivity of ZD1611 for ET Receptors. When tested at a concentration of 10⁻⁴ M, with standard ligand-binding assays, ZD1611 had no significant affinity for the following receptors (derived from whole rat forebrain unless noted otherwise): α adrenoceptors (α1A, α2A), β adrenoceptors (β₁, rat cortex; β₂, rat cerebellum), muscarinic (M₁, rat cortex;
M2, rat cardiac atrium), nicotinic, γ-aminobutyric acid (GABA$_{A}$), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, kainate, N-methyl-D-aspartate, histamine (H$_1$), and 5-hydroxytryptamine (serotonin) (5-HT$_{1A}$, 5-HT$_2$) receptors (data not shown).

Effects of ZD1611 on Responses to ET-1 in Rat Aorta. ET-1 potently contracted rat aorta segments with a mean pD$_2$ value of 8.8 ± 0.2. ZD1611 caused a parallel rightward shift of the CRC to ET-1 without suppression of the maximal response (Fig. 3). Schild analysis yielded a pA$_2$ value for ZD1611 of 7.5 ± 0.3 and a slope of 0.80 ± 0.20 ($n$ = 11; $p$ not significant compared to unity), indicating competitive antagonism (Fig. 3).

In Vivo Potency of ZD1611 in Rats. ZD1611 caused a dose-related rightward shift of the partial dose-response curve to big ET-1 in pithed rats. The threshold dose for significant antagonist activity was established as 0.1 mg/kg (i.v.). An additional effect was observed with 0.3 mg/kg, but this failed to reach statistical significance due to variability within the group (Table 1).

In Vivo Potency of ZD1611 in Dogs. ZD1611 caused a dose-related rightward shift of the partial dose-response curve to big ET-1 in anesthetized dogs at 0.1 and 0.3 mg/kg, i.v. The shift at 0.3 mg/kg was significantly different from control (Table 2).

Reversal of an Established Big ET-1 Pressor Response by ZD1611. An increase in MAP from a mean resting value of 64 ± 1 mm Hg to approximately 100 mm Hg was achieved and maintained following infusion of big ET-1 (0.065 nmol/kg/min) in pithed rats. ZD1611 (0.03–1.0 mg/kg i.v.) caused a dose-related reversal of the big ET-1 pressor response in the presence of a continuous big ET-1 infusion (Fig. 4). The dose of ZD1611 required to produce a 50% reversal of the big ET-1 response was 0.2 (95% CLs = 0.1–0.3) mg/kg.

Activity of ZD1611 against BQ3020-Induced Depressor Responses. The effect of ZD1611 on the depressor response to the selective ET$_B$ agonist BQ3020 was assessed in pithed rats. A small attenuation of the BQ3020 depressor response was observed on administration of vehicle, with a mean dose ratio of 2.3 (95% CLs = 1.7–3.1; $n$ = 4). However, no additional, significant effect ($p$ < .05) was obtained after the infusion of ZD1611 (1.0 mg/kg; mean dose ratio, 3.0; 95% CLs = 1.4–6.1; $n$ = 4), indicating that ZD1611 was without activity at the endothelial ET$_B$ receptor.

Oral Activity of ZD1611 in Conscious Rats. ZD1611 caused dose- and time-dependent antagonism of the pressor response to big ET-1 in pithed rats (Fig. 5). ZD1611 caused a parallel rightward shift of the concentration-response curves to ET-1 in rat isolated aorta. Values are arithmetic means with S.E.M. and are expressed as a percentage of the maximum response to 30 mM barium chloride ($n$ = 11). The slope from Schild regression (inset) was not significantly different from unity.

### TABLE 1

<table>
<thead>
<tr>
<th>ZD1611 (mg/kg, i.v.)</th>
<th>Mean Dose Ratio (95% CLs)</th>
<th>$n$</th>
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<tr>
<td>Vehicle</td>
<td></td>
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<tr>
<td>0.03</td>
<td>1.7 (1.0–2.9)</td>
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<tr>
<td>0.1</td>
<td>2.6 (1.8–4.0)*</td>
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<tr>
<td>0.3</td>
<td>4.9 (1.6–15.6)</td>
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* $p$ < .01 compared with vehicle control.
TABLE 2
Effect of ZD1611 on pressor responses to big ET-1 in anesthetized dogs
Values are mean ratios of the dose of big ET-1 required to give an increase in MAP of 30 mm Hg in the absence and presence of ZD1611.

<table>
<thead>
<tr>
<th>ZD1611 mg/kg, i.v.</th>
<th>Mean Dose Ratio (95% CLs)</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Vehicle</td>
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<tr>
<td>0.1</td>
<td>5.6 (1.1–28.8)</td>
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<tr>
<td>0.3</td>
<td>7.0 (4.5–10.8)*</td>
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</table>

* p < .01 compared with vehicle control.

Fig. 4. Effect of cumulative i.v. ZD1611 (0.03–1 mg/kg; n = 4) and vehicle (n = 5) on established big ET-1-induced pressure responses in pithed rats. Values are arithmetic means with S.E.M. *p < .05; **p < .01 compared with vehicle.

Fig. 5. Duration of action of p.o. administered ZD1611 in conscious rats.
Activity of ZD1611 was calculated as the ratio of the dose of big ET-1 required to give a 30-mm Hg rise in MAP in the absence and then the presence of the compound. ZD1611 was administered either at a dose of 0.3 mg/kg, p.o. (n = 5) and its effect on the big ET-1-induced response tested at 0.5, 2, and 4 h after dosing, or at a dose of 1.5 mg/kg, p.o. (n = 6) when the effect was monitored 0.5, 7, and 10 h after dosing. Values are the geometric mean with 95% CLs. *p < .05; **p < .01; ***p < .001 compared with vehicle.

TABLE 4
Effect of p.o. ZD1611 on pressor responses to big ET-1 in dogs
Values are the geometric mean dose of big ET-1 required to give an increase in MAP of 20 mm Hg. Dogs were treated with either vehicle or p.o. ZD1611, and comparisons were made between animals.

<table>
<thead>
<tr>
<th>Ed50 (95% CLs)</th>
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<tr>
<td>3 h</td>
<td>5 h</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.3 (0.2–0.5)</td>
</tr>
<tr>
<td>ZD1611 (0.6 mg/kg p.o.)</td>
<td>1.3 (1.1–1.5)*</td>
</tr>
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</table>

* p < .001, **p < .05 compared with vehicle control.

Discussion

In the present study, we describe the novel, nonpeptide ETA receptor antagonist ZD1611. ZD1611 has high affinity for human cloned ETA receptors (pIC50 = 8.6) and is 1000-fold selective for human ETA receptors compared with human ETB receptors (pIC50 = 5.6). The potency and selectivity of ZD1611 is similar to that reported for peptide, e.g., FR193917 (IC50 at porcine ETA = 0.53 nM and ETB = 4650 nM; Sogabe et al., 1993) and nonpeptide ET receptor antagonists, including SB217242 (Ki at human ETA = 1.1 nM and at human ETB = 111 nM; Ohlstein et al., 1996), BMS-182874 (Ki at human ETA = 48 nM and at human ETB = >50 μM; Webb et al., 1995), and bosentan (Ki at human ETA = 4.7 nM and at human ETB = 95 nM; Clozel et al., 1994).

The ability of ZD1611 to antagonize ET-induced constrictor responses in vitro was investigated by using the rat isolated aorta, a preparation in which ETA receptor-mediated contractions to ET-1 have been well characterized (Panek et al., 1992; Ohlstein et al., 1994). ZD1611 caused a parallel rightward shift of the CRC to ET-1 without affecting the maximal response, indicating competitive antagonism. Schild analysis of the data yielded a pA2 value of 7.5 ± 0.3. Consistent with the binding data, this pA2 value is comparable to those described for other nonpeptide ETA receptor antagonists such as PD156707 (pA2 = 7.5; Reynolds et al., 1995) and BMS 182874 (Ki at ETA = 520 nM; Webb et al., 1995), and nonselective antagonists including SB217242 (Ki at ETA = 4.4 nM; Ohlstein et al., 1996) and bosentan (pA2 = 7.2; Clozel et al., 1994), although other compounds have higher potency in vitro, e.g., A-127722 (pA2 = 9.2; Opengorth et al., 1996) and SB209670 (pA2 = 9.4; Ohlstein et al., 1994).

The selectivity and potency of ZD1611 observed in vitro
was paralleled in vivo. The pressor response to big ET-1 (which is converted to ET-1 in vivo) appears to be mediated mainly via the ET<sub>A</sub> receptor, although a small ET<sub>B</sub> component has been inferred by some studies (McMurdo et al., 1993; Clozel et al., 1994). ZD1611 effectively blocked the pressor response to big ET-1 in pithed rats. Importantly, ZD1611 exhibited high in vivo potency in two animal species, the rat and the dog, suggesting a nonspecies-dependent action. The threshold i.v. dose of ZD1611 required to block the big ET-1 pressor response was determined as 0.1 and 0.3 mg/kg in the rat and dog, respectively. In contrast, the compound had no significant effect on the depressor response to ET-1 with effective threshold doses of 0.3 and 0.6 mg/kg in conscious rats. In dogs, ZD1611 was active at a slightly higher dose than those required for activity via the i.v. route, indicating good oral bioavailability. ZD1611 was active at a dose of 0.6 mg/kg p.o. for at least 6 h. ZD1611 therefore has a long duration of action when administered p.o., which may allow an infrequent dosing regimen when humans.

In summary, ZD1611 is a selective and potent ET<sub>A</sub> receptor antagonist with functional activity. ZD1611 potently inhibits ET<sub>A</sub>-mediated, ET-1-induced vasoconstriction both in vitro and in vivo but has no action at the dilator ET<sub>B</sub> receptor. In addition, ZD1611 is effective in reversing established ET-1-induced constriction, a profile which may be of clinical importance. ZD1611 appears to be highly bioavailable when administered p.o. and has a long duration of action. These data suggest that ZD1611 may be of therapeutic utility in the treatment of acute or chronic conditions associated with raised levels of ET.

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