Pharmacological Profile of ZD1611, a Novel, Orally Active Endothelin ET_A Receptor Antagonist

CAMPBELL WILSON, SARAH-JANE HUNT, ERIC TANG, NICOLA WRIGHT, ELIZABETH KELLY, SHARON PALMER, CHRISTINE HEYS, SUSAN MELLOR, ROGER JAMES, and RUSSELL BIALECKI

Zeneca Pharmaceuticals, Alderley Park, Macclesfield, United Kingdom

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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_A receptor antagonist ZD1611 [3-(3-methoxy-5-methylpyrazin-2-ylsulfonyl)-2-pyridyl]phenyl]-2,2-dimethylpropionic acid]. ZD1611 competitively inhibited [125I]-labeled ET-1 binding at human cloned ET_A and ET_B receptors with pIC_{50} values of 8.6 ± 0.1 and 5.6 ± 0.1, respectively, showing 1000-fold selectivity for the ET_A 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 in the ET-1-response curve (pA_{2}) 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_B-selective)-induced depressor response in pithed rats indicated a lack of activity at the endothelial ET_B 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 ETA 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 isofrom in the vasculature and elicits a variety of actions including constriction, dilatation (via the release of nitric oxide or prostacyclin), and cell proliferation (Ohlstein and Douglas, 1993; see Masaki, 1995). The ETs mediate their effects via two known receptor subtypes, the ET_A and the ET_B 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., 1990). The receptors may be distinguished pharmacologically, based on the rank order of potency of the ET isoforms, at the ET_A receptor ET-1 > ET-2 > ET-3; and at the ET_B receptor, all three isopeptides are equipotent (Sakurai et al., 1992).

Within the vasculature, ET_A receptors have been localized to smooth muscle cells where they mediate the constrictor and mitogenetic actions of ET (Sakurai et al., 1992; Ohlstein and Douglas, 1993). Constrictor ET_B 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_B receptors has been identified in human vascular smooth muscle (Davenport et al., 1995), the functional relevance of this receptor remains unclear, and the ET_A receptor appears to predominate (Godfraind, 1993; Maguire and Davenport, 1995). ET_B 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), pul monary 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 ET\textsubscript{B} receptor is important given that the ET\textsubscript{B} receptor has been implicated in the clearance of plasma ET-1 (Gasic et al., 1994). Here, we describe the pharmacological profile of ZD1611 [3-{4-[3-(3-methoxy-5-methylpyrazin-2-yl)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 ET\textsubscript{B} receptor.

Materials and Methods

Preparation of Cell Membranes. The cDNA for human ET\textsubscript{A} and ET\textsubscript{B} 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 mouse erythroblastic leukemia (MEL)-C88 cells according to previously described methods (Needham et al., 1992).

MEL-C88 cells identified as expressing cloned human ETA or ET\textsubscript{B} 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 \(\mu\)g/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 \(\mu\)g/ml; bacitracin, 100 \(\mu\)g/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 \(\times\) g for 15 min at 4°C), and membranes were sedimented after centrifugation of the supernatant (40,000 \(\times\) g for 15 min at 4°C). The resultant pellet was resuspended in homogenization buffer and frozen until use.

Radioligand Binding in Cell Membranes. MEL-C88 cell membranes containing either cloned human ETA or ET\textsubscript{B} receptors (1.5 \(\mu\)g of protein per assay well) were incubated with 30 pM \(^{125}\)I-labeled ET-1 in the presence of increasing concentrations of ZD1611 (100 \(\mu\)M to 10 \(\mu\)M), in a final incubation volume of 225 \(\mu\)l. 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. \(^{125}\)I-labeled ET-1 binding was quantified by gamma counting. Nonspecific binding was defined by \(^{125}\)I-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% O\textsubscript{2}/5% CO\textsubscript{2}) physiological salt solution (PSS) composed of 119 mM NaCl, 4.7 mM KCl, 1.6 mM Ca\textsubscript{Cl}\textsubscript{2}, 1.2 mM KH\textsubscript{2}PO\textsubscript{4}, 1.2 mM MgSO\textsubscript{4}, 22.6 mM NaHCO\textsubscript{3}, 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 (Bialkowska and Tulenko, 1989). Briefly, each aortic ring was mounted on two stainless steel pins (130-\(\mu\)m radius) and submerged in a 5-ml organ chamber containing PSS gassed continuously with 95% O\textsubscript{2}/5% CO\textsubscript{2} and maintained at 37°C. The PSS was supplemented with 1 \(\mu\)g propranolol to prevent potential activation of smooth muscle \(\beta\) 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 \(\mu\)M 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 BaCl\textsubscript{2}, 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/cm\textsuperscript{2}), were calculated and analyzed with Bioreport software (Modular Instruments, Malvern, PA).

The concentration of ET-1 required for half the maximal contraction (\(EC_{50}\)) was determined from log-logit plots of individual CRCs and expressed as the negative log of the molar concentration (pD\textsubscript{2}). The concentration of antagonist that caused a 2-fold rightward shift in the ET-1-response curve (pA\textsubscript{2}) was used as an index of potency for a competitive antagonist. Schild plots were constructed and pA\textsubscript{2} values were calculated by linear regression analysis, if the slope did not differ from unity (Arunlakshana 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 respired 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-

\[\text{HO}_2\text{C-}\text{S-}N-\text{O}^\text{H-}\text{OMe}\]

**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 ET<sub>B</sub>-mediated pressor response over the ET<sub>A</sub>-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±10 mm Hg. Two partial dose-response curves to the selective ET<sub>B</sub> receptor agonist BQ3020, separated by a 30-min recovery period, were constructed in the presence of the noradrenaline-induced pressor response. ZD1611 (1.0 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 premedicated with acetylprocainamide (ACP, injection, 2 mg/kg i.v.) and pentobarbital sodium (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 ED<sub>50</sub> 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, benzaminidine, BSA, noradrenaline, phenanthroline, and propranolol were purchased from Sigma Chemical Co. (Poole, UK or St. Louis, MO). Heparin was obtained from C.P. Pharmaceuticals (Wrexham, UK), and penicillin and streptomycin were purchased from Life Technologies (Paisley, UK).125I-labeled ET-1 was purchased from Amersham International plc (Amersham Pharmacia Biotech, UK). Saffan was obtained from Pitman-Moore (Uxbridge, Middlesex, UK) Ltd. and Sagatal was obtained from Rhone Mérieux. Acetylprocainamide was obtained from C-Vet (Leyland, Lancashire, UK). Stock solutions (100 μM) of ET-1 were made by dissolving in distilled H<sub>2</sub>O 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. pIC<sub>50</sub> and pD<sub>2</sub> values (negative log of the IC<sub>50</sub> and EC<sub>50</sub>, respectively) are given as arithmetic mean with S.E.M. Significant differences 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; ED<sub>50</sub> and ED<sub>30</sub>, respectively. Mean dose ratios are expressed as geometric mean with 95% CLs. 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 ET<sub>A</sub>, receptors, ZD1611 competed with 125I-labeled ET-1 in a monophasic manner with a pIC<sub>50</sub> value of 8.6 ± 0.1 (n = 5). ZD1611 was 1000-fold less potent at the human ET<sub>A</sub> receptor with a pIC<sub>50</sub> 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<sup>–4</sup> 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 (α<sub>1A</sub>, α<sub>1B</sub>), β adrenoceptors (β<sub>1</sub>, rat cortex; β<sub>2</sub>, rat cerebellum), muscarinic (M<sub>1</sub>, rat cortex;
M2, rat cardiac atrium), nicotinic, γ-aminobutyric acid (GABA<sub>G</sub>), α-aminobutyric acid (GABA<sub>A</sub>), kainate, N-methyl-D-aspartate, histamine (H<sub>1</sub>), and 5-hydroxytryptamine (serotonin) (5-HT<sub>1A</sub>, 5-HT<sub>2</sub>) 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<sub>2</sub> 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<sub>2</sub> 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).

**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<sub>B</sub> 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 < 0.05) 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<sub>B</sub> receptor.

**Oral Activity of ZD1611 in Conscious Rats.** ZD1611 caused dose- and time-dependent antagonism of the pressor

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**Table 1**

<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>
<td>1.0 (0.7–1.6)</td>
<td>4</td>
</tr>
<tr>
<td>0.03</td>
<td>1.7 (1.0–2.9)</td>
<td>4</td>
</tr>
<tr>
<td>0.1</td>
<td>2.6 (1.8–4.0)*</td>
<td>4</td>
</tr>
<tr>
<td>0.3</td>
<td>4.9 (1.6–15.6)</td>
<td>5</td>
</tr>
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* p < .01 compared with vehicle control.
Effect of oral ZD1611 on pressor responses to big ET-1 in conscious rats. At 0.3 mg/kg p.o., ZD1611 was active for >4 h (Table 3 and Fig. 5), whereas at 1.5 mg/kg p.o., ZD1611 remained active for 7 h after administration (Fig. 5).

**Oral Activity of ZD1611 in Dogs.** The effect of ZD1611 on the pressor response to big ET-1 was investigated at a single dose of 0.6 mg/kg p.o. ZD1611 was active for at least 6 h (Table 4). The dose ratios (doses of big ET-1 required to give a 20-mm Hg increase in MAP in dogs treated with either vehicle or ZD1611) were 4 and 2.2 at 3 and 6 h, respectively.

**Discussion**

In the present study, we describe the novel, nonpeptide ET<sub>A</sub> receptor antagonist ZD1611. ZD1611 has high affinity for human cloned ET<sub>A</sub> receptors (pIC<sub>50</sub> = 8.6) and is 1000-fold selective for human ET<sub>A</sub> receptors compared with human ET<sub>B</sub> receptors (pIC<sub>50</sub> = 5.6). The potency and selectivity of ZD1611 is similar to that reported for peptide, e.g., FR139317 (IC<sub>50</sub> at porcine ET<sub>A</sub> = 0.53 nM and ET<sub>B</sub> = 4650 nM; Sogabe et al., 1993) and nonpeptide ET receptor antagonists, including SB217242 (K<sub>i</sub> at human ET<sub>A</sub> = 1.1 nM and at human ET<sub>B</sub> = 111 nM; Opgenorth et al., 1996), BMS-182874 (K<sub>i</sub> at human ET<sub>A</sub> = 48 nM and at human ET<sub>B</sub> = >50 μM; Webb et al., 1995), and bosentan (K<sub>i</sub> at human ET<sub>A</sub> = 4.7 nM and at human ET<sub>B</sub> = 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 ET<sub>A</sub> 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 pA<sub>2</sub> value of 7.5 ± 0.3. Consistent with the binding data, this pA<sub>2</sub> value is comparable to those described for other nonpeptide ET<sub>A</sub> receptor antagonists such as PD156707 (pA<sub>2</sub> = 7.5; Reynolds et al., 1995) and BMS-182874 (K<sub>i</sub> = 520 nM; Webb et al., 1995), and nonselective antagonists including SB217242 (K<sub>i</sub> = 4.4 nM; Ohlstein et al., 1996) and bosentan (pA<sub>2</sub> = 7.2; Clozel et al., 1994), although other compounds have higher potency in vitro, e.g., A-127722 (pA<sub>2</sub> = 9.2; Opgenorth et al., 1996) and SB209670 (pA<sub>2</sub> = 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 ETA receptor, although a small ETB 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 induced by the selective ETB receptor agonist BQ3020, which acts on endothelial ETB receptors to release nitric oxide and/or prostacyclin (Warner et al., 1989). In humans, ET-induced vasoconstriction is mediated predominantly via the ETA receptor subtype (Godfraind, 1993; Maguire and Davenport, 1995), and the mitogenic properties of ET-1 also appear to be mediated via the ETA receptor (see Ohlstein and Douglass, 1993). Under conditions associated with raised ET levels, the pharmacological profile of ZD1611 would allow for blockade of the deleterious constrictor and mitogenic effects of ET without affecting its beneficial dilator action.

In many clinical situations ET receptor antagonists will be required to reverse established vasoconstriction. Cumulative i.v. administration of ZD1611 produced dose-dependent reversal of an established big ET-1-induced pressor response in the pithed rat with a dose of 0.2 mg/kg being required to reverse the response by 50%. Given the apparently slow dissociation of ET-1 from its binding sites (Hirata et al., 1988; Devadason and Henry, 1997), the mechanism(s) involved in this response is unclear. However, ET receptor internalization, recycling, and subsequent externalization (Marsault et al., 1993) may allow ZD1611 to compete with ET-1 and reverse established and sustained contractions.

An essential characteristic of any compound used for the treatment of chronic disease is good oral potency and duration of action. When administered p.o., ZD1611 caused concentration-dependent blockade of the pressor response to big ET-1 with effective threshold doses of 0.3 and 0.6 mg/kg in conscious rats and dogs, respectively. These doses were only slightly higher than those required for activity via the i.v. route, indicating good oral bioavailability. ZD1611 was active for more than 4 h at a dose of 0.3 mg/kg p.o. and more than 7 h at 1.5 mg/kg p.o. in conscious rats. In dogs, 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 ETA receptor antagonist with functional activity. ZD1611 potently inhibits ETA-mediated, ET-1-induced vasoconstriction both in vitro and in vivo but has no action at the dilator ETB 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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Send reprint requests to: Dr. C. Wilson, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, SK10 4TG, UK.