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Superiority of YM598 over atrasentan as a selective endothelin ET_A receptor antagonist

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Abstract

The binding affinities of (E)-*N*-[6-methoxy-5-(2-methoxyphenoxy)[2,2′ -bipyrimidin]-4-yl]-2-phenylethenesulfonamide monopotassium salt (YM598) for native human endothelin ET_A and ET_B receptors expressed in human coronary artery smooth muscle cells (CASMC) and a human melanoma cell line, SK-Mel-28, respectively, were examined, and the results compared with those for the endothelin receptor antagonists atrasentan and bosentan. The in vivo endothelin ET_A receptor inhibitory activities of YM598 and atrasentan were also compared through the suppression of the big endothelin-1-induced pressor response in pithed rats. K_i values of YM598, atrasentan, and bosentan for native human endothelin ET_A receptors were 0.772, 0.0551, and 4.75 nM, while those for native human endothelin ET_B receptors were 143, 4.80, and 40.9 nM, respectively. The calculated selectivity ratios of YM598, atrasentan, and bosentan for endothelin ET_A versus ET_B receptors were 185, 87 and 8.6, respectively. In pithed rats, YM598 and atrasentan inhibited the big endothelin-1 (1 nmol/kg)-induced pressor response in a dose-dependent manner on both intravenous and oral administration. The inhibitory effect of YM598 was less potent than that of atrasentan when these agents were intravenously administered, but closely similar on oral administration. These results suggest that YM598 has high selectivity for native human ET_A against ET_B receptors, and that YM598 is superior to atrasentan as an ET_A receptor antagonist with regard to pharmacological bioavailability in rats.

Keywords: YM598; Atrasentan; Bosentan; Antagonist; Endothelin receptor

1. Introduction

Endothelin, a potent vasoconstrictive 21-amino acid peptide, was originally isolated from the conditioned medium of cultured porcine vascular endothelial cells in 1988 (Yanagisawa et al., 1988). In the cardiovascular system, endothelin ET_A receptors are expressed abundantly on smooth muscle cells and cardiac myocytes, and mediate contractive and mitogenic actions of endothelin (Sakurai et al., 1992; Ohlstein and Douglas, 1993). In contrast, endothelin ET_B receptors are expressed predominantly on vascular endothelial cells and to a much lesser extent on vascular smooth muscle cells, and cause relaxation of constricted smooth muscle via the release of physiological mediators such as nitric oxide and prostacyclin (Warner et al., 1989). Since the time of their discovery, endothelins have been thought to be implicated in the pathogenesis of various cardiovascular diseases owing to their ability to constrict vascular and nonvascular smooth muscle. This in turn has suggested that endothelin receptor antagonists will be useful in the treatment of cardiovascular diseases such as myocardial infarction, hypertension, heart failure, atherosclerosis, cerebral and coronary vasospasm, renal failure, and asthma (for review, see Rubanyi and Polokoff, 1994; Miyauchi and Masaki, 1999). Several lines of evidence suggest that, among endothelin receptor antagonists, selective endothelin ET_A receptor antagonists may be better than

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non-selective endothelin receptor antagonists in such use. For example, endothelin ET_B receptors are involved in the clearance of endothelin-1 from circulation, and endothelin ET_B receptor blockade causes an increase in plasma endothelin-1 concentration (Fukuroda et al., 1994; Willette et al., 1998). Endothelin ET_B receptor blockade abolishes endothelin ET_B receptor-mediated vasodilation, which is beneficial in the treatment of various cardiovascular diseases (Rubanyi and Polokoff, 1994). In addition, administration of selective endothelin ET_B receptor antagonists in humans produces a decrease in forearm blood flow, which suggests that endothelin ET_B receptor antagonism may cause vasoconstriction (Verhaar et al., 1998).

We have already reported the pharmacological profile of a novel, potent, and orally active selective endothelin ET_A receptor antagonist, (E)-*N*-[6-methoxy-5-(2-methoxyphenoxy)[2,2′ -bipyrimidin]-4-yl]-2-phenylethenesulfonamide monopotassium salt (YM598; Yuyma et al., 2003). In the present study, to confirm the selectivity of YM598 for the endothelin ET_A receptor against the ET_B receptor, we examined the binding profile of YM598 for native human endothelin ET_A and ET_B receptors expressed in human coronary artery smooth muscle cells (CASMC, Davenport et al., 1995; Maguire et al., 1997) and a human melanoma cell line, SK-Mel-28 (Yohn et al., 1994), and compared the results with those for the endothelin receptor antagonists atrasentan (Opgenorth et al., 1996) and bosentan (Clozel et al., 1994), which are under clinical development or available commercially, respectively. Further, to determine pharmacological bioavailability, an important property of drug compounds, we also compared the in vivo endothelin ET_A receptor inhibitory activities of YM598 and atrasentan on oral and intravenous administration through their suppression of the big endothelin-1-induced pressor response in pithed rats.

2. Materials and methods

2.1. Materials and experimental animals

YM598, atrasentan, and bosentan were synthesized at Yamanouchi Pharmaceutical Co., Ltd. (Ibaraki, Japan). [^{125}I]endothelin-1 (specific activity, 2200 Ci/mmol) was obtained from Perkin-Elmer (Boston, MA, USA). Endothelin-1, big endothelin-1, and sarafotoxin S6c were from Peptide Institute (Osaka, Japan). Cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu-) (BQ-123) and [2,6-dimethylpiperidinecarbonyl-γ-methyl-Leu-N_{in}-(methoxycarbonyl)-D-Trp-D-Nle] sodium salt (BQ-788) were from Sigma-Aldrich (St. Louis, USA). CASMC and SK-Mel-28 were from Takara Bio (Shiga, Japan) and the American Tissue Culture Collection (Rockville, MD, USA), respectively. Smooth muscle growth medium (SmBM®) for culturing CASMC was from Takara Bio Inc. Roswell Park Memorial Institute 1640 (RPMI 1640) medium, fetal bovine serum and trypsin-EDTA were from Gibco (Grand Island, NY, USA). All other chemicals were of analytical grade purchased from Wako Pure Chemical Industries (Osaka, Japan).

Male Wistar rats (12–15 weeks of age) were purchased from Japan SLC, (Shizuoka, Japan). During the acclimatization period, solid food and water were provided ad libitum.

2.2. Cell culture

CASMC were cultured at 37 °C in a humidified atmosphere with 5% CO_2 in SmBM® supplemented with 5% fetal bovine serum, 0.5 ng/ml human epithelial growth factor, 2 ng/ml human fibroblast growth factor-B, and 5 μg/ml insulin. SK-Mel-28 were cultured at 37 °C under a humidified 95% air/5% CO_2 atmosphere in RPMI 1640 supplemented with 10% fetal bovine serum.

2.3. Membrane preparation

Plasma membranes were prepared from CASMC and SK-Mel-28. Confluent CASMC or SK-Mel-28 was washed with phosphate-buffered saline (PBS) and harvested into ice-cold 10 mM Tris–HCl, pH 7.4, containing 5 mM EDTA followed by homogenization. Cells lysate was centrifuged at 800×*g* for 10 min to remove unbroken cells and nuclei. The supernatant was centrifuged at 100,000×*g* for 1 h at 4 °C, and the pellet was resuspended in 50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$ and stored in small aliquots which included sufficient protein for assays at −80 °C until use. Protein concentration was determined by the method of Bradford using bovine serum albumin as a standard.

2.4. Binding assay

Endothelin receptor binding assays were performed according to the method of Webb et al. (1993) as described previously with some modifications. Competitive binding studies were performed in a total volume of 250 μl containing 25 μl [^{125}I]endothelin-1 (200 pM), 25 μl competing compounds or 100 nM endothelin-1 to define nonspecific binding, membrane protein of CASMC or SK-Mel-28, and incubation buffer (50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$ and 0.01% bovine serum albumin). The stored membranes were reconstructed by the addition of incubation buffer, and reaction was initiated by the addition of 200 μl of plasma membrane suspension, which contained 10 μg (CASMC) and 5 μg (SK-Mel-28) of membrane protein. After the 3-h incubation period, the reaction was terminated by adding 3 ml of ice-cold incubation buffer followed by rapid filtration through Whatman GF/C filters. The filters were rinsed three times and the radioactivity retained on the filters was counted using a gamma counter at 87% efficiency. We also performed saturation binding studies to yield the dissociation constant (K_d) of the ligand. Each plasma membrane preparation was incubated with various

concentrations of [^{125}I]endothelin-1 (about 20–300 pM) in the absence or presence of 100 nM endothelin-1. Assay conditions were the same as those described for the competitive binding studies.

Maximal specific binding was calculated as total binding minus nonspecific binding. The concentration of test compound that caused 50% inhibition (IC_{50}) of the specific binding of [^{125}I]endothelin-1 was determined by regression analysis of displacement curves. Inhibitory dissociation constant (K_i) was calculated from the following formula: $K_i=IC_{50}/(1+[C]/K_d)$, where $[C]$ is the concentration of radioligand present in the tubes and K_d is obtained from the Scatchard plot (Cheng and Prusoff, 1973).

In the first experiment, we confirmed the main endothelin receptor subtype expressed in CASMC or SK-Mel-28. The binding study was performed using representative endothelin receptor agonists or antagonists: endothelin-1, sarafotoxin S6c (selective endothelin ET_B receptor agonist; Williams et al., 1991), BQ-123 (selective endothelin ET_A receptor antagonist; Ihara et al., 1992), and BQ-788 (selective endothelin ET_B receptor antagonist; Ishikawa et al., 1994).

In the second experiment, we compared the binding affinities of YM598, atrasentan, and bosentan for native human endothelin receptors expressed in CASMC and SK-Mel-28.

2.5. In vivo endothelin ET_A receptor-inhibitory potency: inhibition of pressor response to big endothelin-1 in pithed rats

In vivo antagonistic activity in pithed rats was evaluated according to the method of Clozel et al. (1994) as described previously. Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). After tracheal intubation, they were pithed with a steel rod and artificially ventilated with room air (65 strokes/min; 10 ml/kg) using an artificial respirator (SN-400-7; Shinano Seisakuzyo, Nagano, Japan). A polyethylene cannula for blood pressure measurement (PE-50) filled with heparin (5000 U/ml) was inserted into the left carotid artery. Blood pressure was measured with a pressure transducer (TP-400T; Nihon Kohden, Tokyo, Japan) connected to the carotid arterial cannula and continuously recorded via a polygraph system (Nihon Kohden). Heart rate was measured using the blood pressure pulse wave as trigger. Further, a cannula for drug administration (PE-50) filled with physiological saline was inserted into the left femoral vein. When blood pressure and heart rate were stabilized after a recovery period (about 20 min), YM598 (0.1, 0.3, and 1 mg/kg), atrasentan (0.003, 0.01, 0.03, and 0.1 mg/kg) or the vehicle was intravenously administered. Dosing volume of the test substances and the vehicle was set at 0.5 ml/kg. Approximately 5 min after injection of compounds, big endothelin-1 (1 nmol/kg) was intravenously administered and blood pressure was measured to examine the effects of YM598 and atrasentan on big endothelin-1-induced pressor responses. The change in diastolic blood pressure was used as an index of pressor response.

In another experiment, the oral activity of YM598 and atrasentan was investigated. YM598 (0.3, 1, and 3 mg/kg), atrasentan (0.3, 1, and 3 mg/kg), or 0.5% methyl cellulose as vehicle was orally administered to rats with a dosing cannula. Dosing volume of the test substances and vehicle was set at 5 ml/kg. Approximately 20 min after administration of compounds, the rats were anesthetized with sodium pentobarbital, and then pithed and ventilated 30 min after dosing. Approximately 1 h after oral administration of compounds, big endothelin-1 (1 nmol/kg) was intravenously administered, and blood pressure was measured. In these two experiments, the dose of test compound that caused 50% inhibition (ID_{50}) of the big endothelin-1-induced increase in diastolic blood pressure was determined by linear regression analysis.

In addition, we also investigated the duration of endothelin receptor inhibition after oral administration of YM598 and atrasentan to rats. At 0.5, 2, 6, 12, 18, 24, and 30 h after administration of YM598, atrasentan (3 mg/kg), or 0.5% methyl cellulose, the rats were pithed under anesthesia by pentobarbital. Approximately 30 min after pithing, big endothelin-1 (1 nmol/kg) was intravenously administered, and blood pressure was measured.

2.6. Expression of results

Values are expressed as the mean±S.E.M. or mean value with 95% confidence limits. N represents the number of separate experiments or animals in each group unless otherwise noted. Data were analyzed using the SAS software (SAS Institute, NC, USA). The difference between groups was analyzed by the unpaired Student's t-test for two-group comparison, and by Dunnett's or Tukey's multiple comparison test for comparisons of more than two groups. A P value less than 0.05 was considered significant.

2.7. Ethical considerations

The protocol for this study was approved by the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd.

3. Results

3.1. Confirmation of main endothelin receptor subtype expressed in CASMC or SK-Mel-28

The presence of endothelin receptors in plasma membrane prepared from CASMC and SK-Mel-28 was confirmed from the results of saturation binding studies using [^{125}I]endothelin-1. K_d and B_{max} values for [^{125}I]endothelin-1 in CASMC were 22.2±8.69 pM and 115±4.85 fmol/mg protein, and those in SK-Mel-28 were 28.6±8.66 pM and 340±3.10 fmol/mg protein, respectively. In the plasma

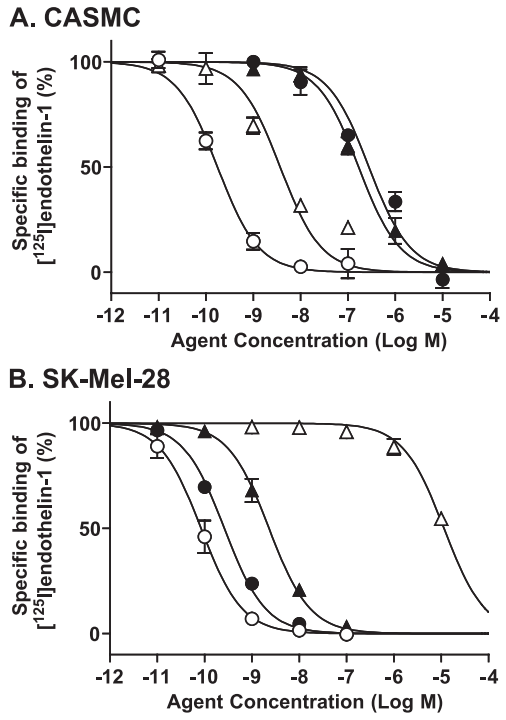

Fig. 1. Binding properties of representative endothelin receptor agonists and antagonists to endothelin receptors expressed in human coronary artery smooth muscle cells (CASMC) and human melanoma SK-Mel-28. Inhibition of specific binding of [^{125}I]endothelin-1 to endothelin receptors expressed in CASMC (A) or SK-Mel-28 (B) by endothelin-1 (○), sarafotoxin S6c (●), BQ-123 (△) and BQ-788 (▲). Each point represents the mean±S.E.M. (n=3). Results are expressed as a percentage of specific binding in the absence of unlabeled agents.

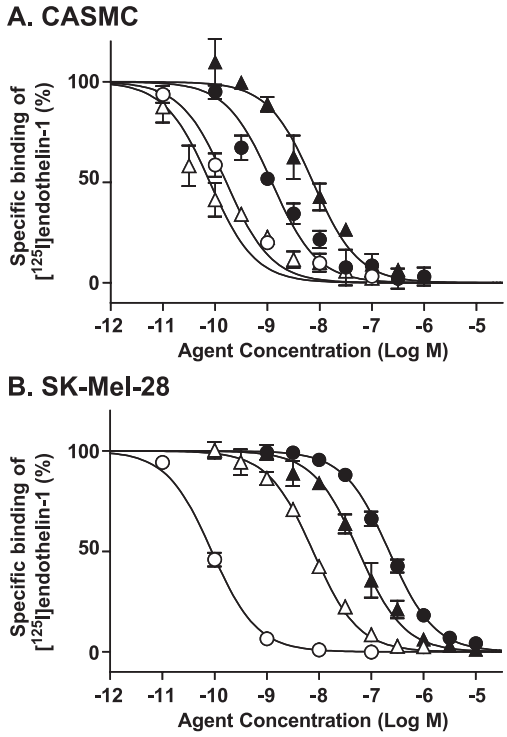

Fig. 2. Binding properties of YM598 and endothelin receptor antagonists to endothelin receptors expressed in human coronary artery smooth muscle cells (CASMC) and human melanoma SK-Mel-28. Inhibition of specific binding of [^{125}I]endothelin-1 to endothelin receptors expressed in CASMC (A) or SK-Mel-28 (B) by YM598 (●), atrasentan (△), and bosentan (▲). Endothelin-1 (○) was used as positive control. Each point represents the mean±S.E.M. (n=4). Results are expressed as a percentage of specific binding in the absence of unlabeled agents.

membrane of CASMC and SK-Mel-28, endothelin-1, sarafotoxin S6c, BQ-123, and BQ-788 inhibited the specific binding of [^{125}I]endothelin-1 in a concentration-dependent manner, and the inhibiting curves were fitted to one-site competition, suggesting the expression of a single subtype of endothelin receptors both in CASMC and SK-Mel-28 (Fig. 1). The rank order of K_i values of these agents in CASMC was endothelin-1>BQ-123>BQ-788≥sarafotoxin S6c, suggesting the expression of endothelin ET_A receptors (Table 1). In contrast, the rank order of K_i values of these agents in SK-Mel-28 was endothelin-1>sarafotoxin S6c>BQ-788 ⩾ BQ-123, suggesting the expression of endothelin ET_B receptors (Table 1).

Table 1
Affinities of representative endothelin receptor agonists and antagonists for endothelin receptors expressed in human coronary artery smooth muscle cells (CASMC) or human melanoma SK-Mel-28

	K_i (nM)	
	CASMC	SK-Mel-28
Endothelin-1	0.110±0.0371	0.0506±0.00786
Sarafotoxin S6c	188±77.9	0.166±0.0366
BQ-123	2.30±0.807	>10,000
BQ-788	106±30.5	1.40±0.223

Data represent the mean±S.E.M. (n=3). Abbreviations: K_i=affinity constant.

3.2. Affinities of YM598 and endothelin receptor antagonists for native human endothelin receptors expressed in CASMC and SK-Mel-28

In the plasma membrane of CASMC and SK-Mel-28, YM598, atrasentan, and bosentan inhibited the specific binding of [^{125}I]endothelin-1 in a concentration-dependent manner (Fig. 2). K_i values of each agent for endothelin receptors in CASMC were as follows: YM598: 0.772±

Table 2
Affinities of YM598 and endothelin receptor antagonists for endothelin receptors expressed in human coronary artery smooth muscle cells (CASMC) or human melanoma SK-Mel-28

	K_i (nM)		
	CASMC (ET_A)	SK-Mel-28 (ET_B)	ET_A selectivity
YM598	0.772±0.181	143±20.7	185
Atrasentan	0.0551±0.0267	4.80±0.314	87
Bosentan	4.75±1.44	40.9±12.3	8.6

Data represent the mean±S.E.M. (n=4). Abbreviations: K_i=affinity constant. ET_A=endothelin ET_A receptor. ET_B=endothelin ET_B receptor. Endothelin ET_A receptor selectivity was calculated by dividing the affinity in SK-Mel-28 by that in CASMC.

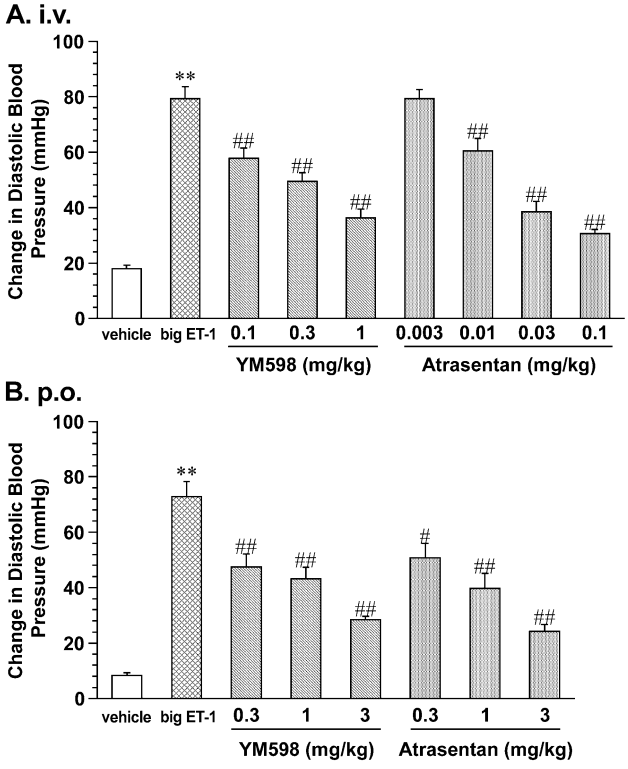

Fig. 3. Effects of intravenous (A) and oral (B) administration of YM598 and atrasentan on big endothelin-1 (big ET-1; 1 nmol/kg, i.v.)-induced pressor responses in pithed rats. Each column represents the mean±S.E.M. of six experiments. $^{**}P<0.01$ compared with corresponding vehicle for big ET-1 value tested by the unpaired Student's *t*-test, $^{\#}P<0.05$; $^{\#\#}P<0.01$ compared with the corresponding big ET-1 value tested by Dunnett's multiple range test.

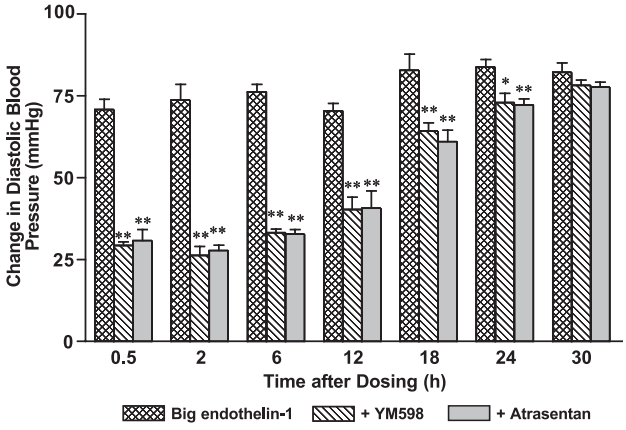

Fig. 4. Inhibitory effects of YM598 and atrasentan on the big endothelin-1 (1 nmol/kg, i.v.)-induced pressor responses in diastolic blood pressure in pithed rats after oral administration. Rats were pithed at 0.5, 2, 6, 12, 18, 24 or 30 h after oral administration of vehicle (0.5% methyl cellulose), YM598, or atrasentan. Each column with vertical bar represents the mean±S.E.M. of six experiments. $^{*}P<0.05$, $^{**}P<0.01$ compared with corresponding big endothelin-1 value tested by Tukey's multiple range test. Statistical significance between YM598 and atrasentan values was not observed at any experimental point.

0.181 nM; atrasentan: 0.0551±0.0267 nM; and bosentan: 4.75±1.44 nM, and those in SK-Mel-28 were as follows: YM598: 143±20.7 nM; atrasentan: 4.80±0.314 nM; and bosentan: 40.9±12.3 nM, respectively (Table 2). The calculated selectivity ratios of YM598, atrasentan, and bosentan for endothelin ET_A versus ET_B receptors were 185, 87, and 8.6, respectively (Table 2).

3.3. Inhibitory effects of YM598 and atrasentan on big endothelin-1-induced pressor response in pithed rats

The intravenous injection of big endothelin-1 (1 nmol/kg) induced pressor responses in pithed rats, with an increase in diastolic blood pressure of about 80 mm Hg (Fig. 3). Intravenous (Fig. 3A) or oral (Fig. 3B) administration of YM598 and atrasentan dose-dependently and significantly suppressed these big endothelin-1-induced pressor responses. ID_{50} values of YM598 were 0.287 mg/kg i.v. and 0.869 mg/kg p.o., giving a calculated intravenous/oral dose ratio of 0.330. In contrast, ID_{50} values of atrasentan were 0.020 i.v. and 0.787 mg/kg p.o., with a calculated intravenous/oral ratio of 0.026 (Table 3). The inhibitory effect of YM598 after intravenous administration was approximately 0.07-fold that of atrasentan, and 0.9-fold after oral administration (Table 3). In the experiment on the duration of endothelin receptor inhibition, oral administration of YM598 (3 mg/kg) produced approximately 60% inhibition of the big endothelin-1 (1 nmol/kg)-induced pressor response at 0.5 h after dosing, and approximately 40% inhibition was sustained for at least 12 h (Fig. 4). Moreover, the inhibition was significant even at 24 h after oral administration. Oral administration of atrasentan (3 mg/kg) also inhibited the pressor response induced by big endothelin-1 (1 nmol/kg) (Fig. 4). No statistically signifi-

Table 3
ID_{50} values and the ratio of ID_{50} (intravenous) and ID_{50} (oral) of YM598 or atrasentan, and potency ratio of YM598 against atrasentan in pithed rats

Compound	ID_{50} (mg/kg)		Intravenous/oral
	Intravenous	Oral	
YM598	0.287 (0.172 – 0.458)	0.869 (0.412 – 1.64)	0.330 (0.105 – 1.11)
Atrasentan	0.020 (0.013 – 0.028)	0.787 (0.404 – 1.32)	0.026 (0.010 – 0.070)
Potency ratio of YM598 against atrasentan	0.066 (0.041 – 0.098)	0.859 (0.593 – 1.19)	

Data represent the mean with 95% confidence interval in parentheses. The dose of YM598 and atrasentan that caused 50% inhibition (ID_{50}) of the big endothelin-1-induced increase in diastolic blood pressure was determined by linear regression analysis. The ratio of ID_{50} (intravenous) and ID_{50} (oral) was calculated by dividing the ID_{50} (intravenous) value by ID_{50} (oral) value, and the potency ratio of YM598 was calculated by dividing the ID_{50} value of atrasentan by that of YM598.

cant differences were observed between the inhibitory activities of the two compounds.

4. Discussion

We recently reported the pharmacological properties of a novel, potent, and orally active selective endothelin ET_A receptor antagonist, YM598 (Yuyma et al., 2003). A number of endothelin receptor antagonists have been discovered and put into clinical development. Among them, bosentan, the first orally active endothelin receptor antagonist discovered, has already been launched for the treatment of primary pulmonary hypertension (Rubin et al., 2002), while atrasentan is reported to be one of the most potent selective endothelin ET_A receptor antagonists and is now under clinical development (Carducci et al., 2003). The main purpose of the present study was to compare the in vitro endothelin ET_A receptor selectivity and in vivo pharmacological bioavailability of YM598 with those of atrasentan and bosentan. The results have clarified the superiority of YM598 over existing endothelin ET_A receptor antagonists.

Given that Flynn et al. (2002) reported that the endothelin receptor antagonist sitaxsentan showed different affinities between native and recombinant endothelin ET_A receptors, the present study investigated the affinity of these three compounds for human endothelin receptor using native, not recombinant, endothelin ET_A and ET_B receptors expressed in human coronary artery smooth muscle cells (CASMC) and human melanoma cell line SK-Mel-28. These two cell types are reported to mainly express endothelin ET_A and ET_B receptors, respectively (Davenport et al., 1995; Maguire et al., 1997; Yohn et al., 1994). We confirmed these findings through binding experiments using representative endothelin receptor agonists and antagonists. The affinity of endothelin-1 for human endothelin ET_A receptors is more than 1000-fold greater than that of sarafotoxin S6c with selective ET_B receptor agonistic activity, whereas their affinities for human endothelin ET_B receptors are almost the same (Williams et al., 1993). In the present study, the affinity of endothelin-1 for endothelin receptors in CASMC was 1700-fold higher than that of sarafotoxin S6c, while their affinities for endothelin receptors in SK-Mel-28 were closely similar, indicating that these cells mainly expressed endothelin ET_A and ET_B receptors, respectively. These results are supported by the findings that the selective endothelin ET_A receptor antagonist BQ-123 (Ihara et al., 1992) showed higher affinity for endothelin receptors in CASMC, whereas the selective endothelin ET_B receptor antagonist BQ-788 (Ishikawa et al., 1994) had higher affinity for SK-Mel-28. K_i values of YM598, atrasentan and bosentan for human recombinant endothelin ET_A and ET_B receptors were reported as follows: YM598, 0.697 and 569 nM; atrasentan, 0.034 and 63.3 nM; and bosentan, 6.5 and 343 nM, respectively (Yuyma et al., 2003; Clozel et al., 1994; Opgenorth et al., 1996). In the present study, K_i values of these three antagonists for CASMC were similar to those for recombinant endothelin ET_A receptors, whereas those for SK-Mel-28 were 4- to 10-fold higher than those for recombinant endothelin ET_B receptors. Approximately 10-fold differences in affinity for endothelin receptor antagonists were reported for endothelin receptors from different membrane preparations (Clozel et al., 1994). It is therefore considered that the affinities of YM598, atrasentan and bosentan for native endothelin ET_A and ET_B receptors essentially confirms their affinities for recombinant endothelin ET_A and ET_B receptors. From the direct comparison of the present study, we confirmed that the affinity of YM598 for native endothelin ET_A receptors was between those of atrasentan and bosentan, but that its selectivity for endothelin ET_A receptors was closely similar to or greater than that of atrasentan.

We also investigated the in vivo endothelin receptor-inhibiting activity of YM598. We have already reported the superiority of the in vivo endothelin receptor-inhibiting activity of YM598 compared with that of bosentan (Yuyma et al., 2003), so here we compared the activities of YM598 and atrasentan. On intravenous administration, the inhibitory effect of YM598 on big endothelin-1-induced pressor responses in pithed rats was 0.07-fold that of atrasentan. These results are considered to reflect the difference in the affinity of these two compounds for endothelin ET_A receptors as observed in the binding study. In contrast, the inhibitory effect of YM598 was similar to that of atrasentan on oral administration. The inhibitory duration of these two compounds was also similar. The calculated intravenous/oral dose ratio of YM598 was much higher than that of atrasentan. We have already reported the high oral activity of YM598 in rats (Yuyma et al., 2003), the present results provide further evidence that YM598 may be easily absorbed on oral administration, and moreover with a higher oral bioavailability than atrasentan.

In conclusion, YM598 has a high selectivity for native human ET_A receptors against ET_B receptors, and good bioavailability in rats. These results suggest that YM598 is superior to atrasentan as an endothelin ET_A receptor antagonist from the standpoint of pharmacological bioavailability. They also indicate its potential role as a new tool for use in the analysis of the pathophysiological role of endothelin ET_A receptors in the various disorders in which endothelins are implicated.

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