Pharmacology of a non-selective ET_A and ET_B receptor antagonist, TAK-044 and the inhibition of myocardial infarct size in rats

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1 The aims of the present study were to characterize the pharmacological profile of a new endothelin (ET) receptor antagonist, TAK-044 and to consider whether it limits the extension of myocardial infarct size in rats.

2 Binding of [¹²⁵I]-ET-1 to ET receptors on rabbit ventricular and cerebellar membrane fractions was inhibited by TAK-044 with IC₅₀ values of 3.8 nM and 130 nM, respectively.

3 It inhibited ET-1, ET-2 and ET-3-induced vasoconstriction of porcine isolated coronary arteries in a competitive (ET-1, ET-2) and a non-competitive (ET-3) manner.

4 In the rat *in vivo*, the ET-1-induced blood pressure changes including transient hypotension followed by sustained hypertension, were inhibited by TAK-044 (0.1–10 mg kg⁻¹, i.v.) in a dose-dependent manner.

5 Acute myocardial infarction induced by 1 h coronary occlusion followed by 24 h reperfusion in rats caused an infarct size of 60 ± 2% (*n* = 12) of the area-at-risk by weight.

6 Intravenous injection of TAK-044 10 min before coronary occlusion reduced the infarct size in a dose-dependent manner: 32% and 54% reductions at 1 and 3 mg kg⁻¹, respectively.

7 TAK-044 administered 10 min before or 1 h after reperfusion (1 mg kg⁻¹, i.v.) showed similar inhibitory effects: 34% and 23% reductions, respectively.

8 We conclude that TAK-044 is an ET_A/ET_B receptor antagonist which shows strong inhibitory effects on the extension of myocardial infarct size after coronary artery occlusion-reperfusion in rats.

Keywords: Endothelin receptor antagonist; TAK-044; acute myocardial infarction; porcine coronary artery contraction; rat blood pressure; ET_A and ET_B receptors

Introduction

Since the first report of endothelin (ET) and its isoforms, ET-1, ET-2 and ET-3, one of the main research issues has been to characterize their pathophysiological role in diseases. Various clinical and animal studies have addressed this (Masaki *et al.*, 1992; Haynes & Webb, 1993 for review) and have indicated that the endothelin, especially ET-1 is involved in diseases such as acute myocardial infarction (Miyachi *et al.*, 1989; Watanabe *et al.*, 1990; 1991), acute renal failure (Tomita *et al.*, 1989; Shibouta *et al.*, 1990), subarachnoid haemorrhage (Masaoka *et al.*, 1989) and hypertension (Saitoh *et al.*, 1990). Contribution of endogenous endothelin in these conditions was justified mainly on the basis of increased plasma ET-1 levels in patients and animal disease models compared with those in normal subjects. Additional indirect evidence was provided by studies on the effects of exogenous endothelin *in vivo* and *in vitro* and on the depletion of endogenous ET-1 levels by a monoclonal antibody in animal models (Shibouta *et al.*, 1990; Watanabe *et al.*, 1991). However, since the changes in plasma endothelin contents were found to be minimal in most studies, more definitive evidence for the involvement of endothelin in pathophysiological processes, such as results of studies using specific endothelin antagonists have been awaited to clarify the issue.

In addition, understanding of the pathophysiological roles of endothelins is complicated by the existence of ET receptor subtypes, ET_A (Arai *et al.*, 1990) and ET_B (Sakurai *et al.*, 1990). These receptor subtypes showed different sensitivities

to the three endothelin isoforms so that ET-1 and ET-2 had high, almost equal, affinities for both ET_A and ET_B receptors (Sakurai *et al.*, 1990), whereas ET-3 had a higher affinity for ET_B than ET_A receptors (Arai *et al.*, 1990). Although ET_A and ET_B receptors were originally assigned to be responsible for ET-1-induced vasoconstriction and vasorelaxation, respectively (Masaki *et al.*, 1992), it has been demonstrated that both receptor subtypes were involved in ET-1-induced vasoconstriction (Sumner *et al.*, 1992; Clozel *et al.*, 1992) as well as hypertension (McMurdo *et al.*, 1993). Since increases in ET-1 levels played an important pathophysiological role in various diseases (e.g. Watanabe *et al.*, 1991), it is reasonable to speculate that both ET_A and ET_B receptors are involved in such conditions.

The recent availability of specific endothelin receptor antagonists, such as BQ-123 (Ihara *et al.*, 1992), FR139317 (Sogabe *et al.*, 1993), IRL1038 (Urade *et al.*, 1992) and Ro 46-2005 (Clozel *et al.*, 1993) and the discovery of a specific ET_B receptor agonist, sarafotoxin S6c (Williams *et al.*, 1991) made it possible to investigate some of the important physiological and pathophysiological roles of endothelins and their receptors described above. Animal studies with endothelin antagonists demonstrated the improvement of functional deterioration induced by acute renal failure (Mino *et al.*, 1992) and by subarachnoid haemorrhage (Clozel *et al.*, 1993) and suggested the involvement of ET_A receptor activation in these diseases. There is, however, little information available as to whether an ET receptor antagonist is useful for treating acute myocardial infarction. Recent animal studies using ET_A antagonists showed controversial results; BQ-123 reduced the acute myocardial infarct size in dogs

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(Grover *et al.*, 1993), whereas FR139317 did not in rabbits (McMurdo *et al.*, 1994).

The present study was, therefore, designed to address the issue whether endothelin receptor antagonists limit the infarct size in rats by use of a newly developed antagonist, TAK-044, cyclo[D- α -aspartyl-3](4-phenylpiperazin-1-yl)carbonyl]-L-alanyl-L- α -aspartyl-D-2-(2-thienyl)glycyl-L-leucyl-D-tryptophyl] disodium (Kikuchi *et al.*, 1994; Kusumoto *et al.*, 1994). First the pharmacological profile of TAK-044 was characterized and it was found to be an ET_A/ET_B receptor antagonist. Effects of TAK-044 on acute myocardial infarction in rats were, then, studied to demonstrate its limiting effects on the extension of infarct size.

Methods

Receptor binding

Cardiac and brain membrane fractions were prepared by the method described previously (Glossmann & Ferry, 1985) with minor modifications. Briefly, ventricular and cerebellar tissues were isolated from New Zealand White rabbits (2.5–3.5 kg), minced and homogenized with a Polytron homogenizer (3/4 maximum speed, 4 times for 10 s each) in 20 mM NaHCO₃ solution containing 0.1 mM phenylmethyl sulphonyl fluoride (PMSF). The homogenates were centrifuged at 15,000 *g* for 15 min and the resulting supernatants were centrifuged twice at 45,000 *g* for 15 min. The final pellets of ventricular and cerebellar membrane preparations were each suspended in 50 mM Tris/HCl (pH 7.4) containing 0.1 mM PMSF and stored in a deep freezer (–80°C) until required for use. The protein contents were determined by the method of Lowry *et al.* (1951). All procedures were carried out at –4°C.

Membrane fractions of ventricle (20 μ g of protein) or cerebellum (5 μ g of protein) were diluted with the assay solution (50 mM Tris/HCl, pH 7.4, 0.2% bovine serum albumin) to make up a final volume of 300 μ l. An aliquot of each diluted membrane fraction was incubated with [¹²⁵I]-ET-1 (60 pM, Amersham) for 90 min at 37°C. The mixture was, then, diluted with chilled assay solution and filtered through a glass fiber filter (GF/B, Whatmann). The radioactivity on each filter was counted with a gamma counter to determine the amount of bound [¹²⁵I]-ET-1. The non-specific binding was determined in the presence of 0.4 μ M ET-1 and the specific binding was defined as the difference between the total and non-specific binding.

Porcine coronary artery preparation

Porcine hearts were obtained from a local slaughter house, and the left anterior descending coronary arteries were isolated to make ring preparations (2–3 mm in length). They were mounted for isometric recording with a resting tension of 2 g in a 20 ml bath containing modified Krebs solution (95% O₂, 5% CO₂, pH 7.4, 36°C), the composition of which in mM was: NaCl 113, KCl 4.6, CaCl₂ 1.2, NaHCO₃ 22, NaH₂PO₄ 3.5, MgCl₂ 1.2 and dextrose 10. The experiments began after a 60 min equilibration period followed by a challenge with 60 mM KCl to obtain a standard vasoconstriction for each preparation. The contractions were measured with a transducer (FD pickup, NEC-Sanei). ET-1, ET-2 and ET-3 (Peptide Institute) was added to the bath solution in a cumulative manner to produce concentration-response curves with and without TAK-044 pretreatment (30 min before the addition of each endothelin).

Endothelium-dependency

A pair of porcine coronary arteries was prepared, one for endothelium-intact and the other for denuded preparations, the latter being prepared by removing the endothelial cells by

gentle rubbing with a cotton bud. Existence of endothelial cell was examined by applying substance P (3 nM) before starting the experiments. Concentration-response curves for ET-1 were obtained using these paired preparations with and without TAK-044 pretreatment (10 nM, 30 min before the addition of ET-1).

An EC₅₀ value was obtained from the concentration-response curve of each preparation, and a pA₂ value was calculated from a Schild plot of the EC₅₀ values, when necessary.

Blood pressure measurements in rats

Male Wistar rats (250–350 g) were anaesthetized with 5-sec-butyl-5-ethyl-2-thiobarbituric acid (inactin, 120 mg kg^{–1}, i.p.). The right femoral artery and right femoral vein were cannulated with polyethylene tubes and another cannula was inserted in the bronchi to ensure respiration was maintained. After a stabilizing period of 30 min, the arterial blood pressure was measured with a transducer (Spectramed) from the femoral arterial cannula. ET-1 was injected via the femoral venous cannula two to three times with 1 h intervals between injections until stable responses in blood pressure were obtained. TAK-044 was, then, injected intravenously 10 min before the next ET-1 administration to determine its inhibitory effects.

Rat acute myocardial infarction model

Male Wistar rats (250–300 g) were anaesthetized with pentobarbitone sodium (50 mg kg^{–1}, i.p.) and the chest was opened to expose the heart. Acute myocardial infarction was induced by coronary artery occlusion for 1 h with reperfusion of the coronary blood flow for 24 h. The infarct size was determined histochemically 24 h after reperfusion as follows. Each rat was anaesthetized again 24 h after reperfusion and the heart was quickly removed. The coronary artery was ligated at the same position as the occlusion and Evans blue dye (1% w/v) was perfused through the aorta to determine the area-at-risk region. Since rat hearts showed less-developed collaterals (Tillmanns & Kübler, 1984), the area-at-risk determined by this method represents the ischaemic region during the arterial occlusion. The infarct regions were determined by incubating sliced tissues with 1% triphenyltetrazolium chloride solution (37°C) for 10 min. The undyed infarct zone was carefully cut out and weighed. The infarct size was expressed as a percentage of the area-at-risk for each heart.

All the data in the present study were expressed as means $\pm$ s.e. with the number of experiments involved. A one-way analysis of variance or Student's *t* test was performed for statistical analysis.

Chemicals

ET-1, ET-2, ET-3 and TAK-044 were dissolved in distilled water for the *in vitro* experiments and in saline for the *in vivo* experiments. TAK-044, BQ 123 (cyclo(-D-Asp-L-Pro-D-Val-L-Leu-D-Trp)) and inactin were synthesized in our chemistry division. 5-Hydroxytryptamine (5-HT), histamine, acetylcholine and substance P were dissolved in distilled water and U-46619 was in ethanol until required for use. All these agents were obtained from Sigma except for substance P (Peptide Institute).

Results

Binding of [¹²⁵I]-ET-1 to rabbit membrane fractions was displaced by both ET-1 and ET-3 although their inhibitory selectivities on ventricular and cerebellar preparations differed. ET-1 displaced the bound [¹²⁵I]-ET-1 from ventricular tissue with an IC₅₀ value of 0.11 $\pm$ 0.04 nM and ET-3

showed weaker potency with an IC_{50} value of 11 ± 4 nM ($n = 4$ each, Figure 1a). In contrast, ET-1 and ET-3 showed similar displacement potencies on cerebellar membrane fractions: IC_{50} values of 0.22 ± 0.05 nM for ET-1 and 0.071 ± 0.02 nM for ET-3 ($n = 4$ each, Figure 1b).

The specific ET_A receptor antagonist, BQ-123, displaced the [125 I]-ET-1 bound more potently from ventricular than cerebellar preparations (Figure 1a,b). The IC_{50} value for the ventricle was 0.18 ± 0.01 μ M, which was 222 times more potent than that for the cerebellum (IC_{50} of 40 ± 7 μ M, $n = 4$ each). The difference in the IC_{50} values between the two preparations with TAK-044 was smaller than that with BQ-123. It displaced the bound [125 I]-ET-1 from the ventricular preparation with an IC_{50} value of 3.8 ± 1 nM ($n = 4$), a potency being 1/35 of ET-1 and 47 times of BQ-123 (Figure 1a). The IC_{50} value of TAK-044 for the cerebellar preparations was 130 ± 28 nM ($n = 4$, Figure 1b).

The effects of TAK-044 on ET-induced vasoconstriction were studied in porcine coronary arteries. ET-1, ET-2 and ET-3 showed strong vasoconstriction in coronary arteries. The concentration-response curve for ET-1 was similar to that for ET-2 (Figure 2a,b). Maximum responses to ET-1 and ET-2 were obtained with 0.1 μ M and their amplitudes were $169 \pm 8\%$ and $176 \pm 32\%$ (%control of the 60 mM KCl-induced standard contraction, $n = 4$), respectively. EC_{50} values for the peptides were obtained from the concentration-response curves: 5.7 nM for ET-1 and 8.8 nM for ET-2. In contrast, the concentration-response curve for ET-3 showed two distinct phases (Figure 2c). Although the threshold concentration and shape of the concentration-response curve up

to 10 nM ET-3 were similar to those of the other two endothelins, the curve between 10 and 300 nM was shallower than for the others.

TAK-044 shifted the concentration-response curves for these three peptides. Pretreatment with TAK-044 inhibited ET-1-induced vasoconstriction in a concentration-dependent manner from 10 nM (Figure 2a). The concentration-response curves were shifted to the right without changing the maximum response, which indicates that the inhibition was competitive: a pA_2 value was calculated to be 8.4. The potency of inhibition for ET-2-induced vasoconstriction was similar to that for ET-1. TAK-044 at 10 nM shifted the ET-2 response curve to the right without changing the maximum response (Figure 2b). In contrast, TAK-044 inhibited ET-3-induced vasoconstriction in a non-competitive manner (Figure 2c). The degree of inhibition was much greater with higher concentrations than with lower concentrations. The selective ET_A receptor antagonist, BQ-123 at 10 μ M, inhibited the second phase of ET-3-induced vasoconstriction only, whereas ET-1-

Figure 1 Binding displacement curves of [125 I]-endothelin-1 ([125 I]-ET-1) by the peptides, ET-1 (O), ET-3 (Δ), BQ-123 ($\blacktriangle$) and TAK-044 ($\bullet$) in rabbit ventricular (a) and in cerebellar membrane fractions (b). Each point represents mean $\pm$ s.e., $n = 4$.

Figure 2 Concentration-response curves for endothelin-1 (ET-1) (a), ET-2 (b) and ET-3 (c) of porcine isolated coronary arteries. Each point represents mean $\pm$ s.e., $n = 4$. Control (O), and TAK-044: 1 ($\bullet$), 10 (Δ), 30 ($\blacktriangle$) and 100 ($\square$) nM.

and ET-2-induced vasoconstriction was inhibited in a competitive manner (data not shown).

The specificity of inhibition by TAK-044 was studied by use of various vasoactive agents. U-46619, 5-HT, acetylcholine, histamine and KCl caused vasoconstriction in porcine coronary arteries with EC_{50} values of 11 nM, 380 nM, 540 nM, 2.6 μ M and 32 mM ($n = 4$ each), respectively. Pretreatment with 10 nM TAK-044 did not alter these concentration-response curves; the respective EC_{50} values were 12 nM, 340 nM, 520 nM, 2.5 μ M and 28 mM ($n = 4$ each). Next, the endothelium-dependency of the inhibitory effects of TAK-044 was studied in coronary arteries. The concentration-response curves for ET-1 with and without TAK-044 were not affected by the presence of the endothelium. The EC_{50} values ($n = 4$ each) for the endothelium-intact and denuded preparations in the absence of TAK-044 were 5.7 nM and 4.0 nM, respectively, and 21 nM for intact and 14 nM for denuded preparations after pretreatment with TAK-044 (10 nM). Finally, application of high concentrations of TAK-044 up to 100 μ M induced neither relaxation nor vasoconstriction in coronary artery preparations ($n = 4$), which indicates that it had no agonist or non-selective effects.

The effects of TAK-044 on blood pressure changes induced by ET-1 were studied *in vivo*. Injection of ET-1 (0.3 nmol kg^{-1} , i.v.) evoked transient hypotension followed by sustained hypertension, which lasted for over 30 min. The maximum decrease in mean blood pressure was 13 ± 4 mmHg and the maximum increase was 24 ± 4 mmHg ($n = 9$, Figure 3). The mean blood pressure before drug injection was 104 ± 3 mmHg ($n = 9$). Pretreatment with TAK-044 (10 min before ET-1 injection) inhibited both these responses in a dose-dependent manner (Figure 3). The maximum hypertension was decreased significantly by 42% at 0.1 $mg\ kg^{-1}$, 50% at 1 $mg\ kg^{-1}$ and 88% at 10 $mg\ kg^{-1}$. Similarly, the maximum hypotension was decreased significantly by 19% at 0.1 $mg\ kg^{-1}$ and by over 90% at 1 and 10 $mg\ kg^{-1}$. It should be noted that injection of TAK-044 (0.01–10 $mg\ kg^{-1}$, i.v.) alone did not modify the resting blood pressure.

One of the main objectives of the present study was to determine whether TAK-044 limited the extension of myocardial infarct size in rats. Coronary artery occlusion for 1 h and reperfusion for 24 h produced myocardial infarction in rats. The infarct size measured after this occlusion and reperfusion of the coronary artery was $60 \pm 2\%$ of the area-at-risk by weight ($n = 12$, Saline control in Figure 4). Pretreatment with

TAK-044 reduced the infarct size in a dose-dependent manner. Intravenous injection of 0.3 $mg\ kg^{-1}$ 10 min before coronary occlusion inhibited the extension of infarct size significantly by 20% compared with the saline controls ($48 \pm 4\%$ of the area-at-risk, $n = 6$, Figure 4). Reductions of 32% and 54% were obtained with 1 and 3 $mg\ kg^{-1}$, i.v., respectively: $41 \pm 3\%$ ($n = 7$) for 1 $mg\ kg^{-1}$ and $28 \pm 6\%$ (% of the area-at-risk, $n = 5$) for 3 $mg\ kg^{-1}$. The area-at-risk of the saline control was $49 \pm 2\%$ of the left ventricular mass by weight and was not altered significantly by drug administration.

Another set of experiments was designed to study the effects of post-treatments with TAK-044 on the infarct size in rats. When TAK-044 was administered 50 min after coronary artery occlusion (10 min before reperfusion started), the resultant infarct size measured 24 h after reperfusion of coronary flow was reduced to $51 \pm 2\%$ ($n = 5$) and $40 \pm 2\%$ (% of the area-at-risk, $n = 12$) at 0.3 and 1 $mg\ kg^{-1}$, i.v., respectively (Figure 5). These reductions were similar to those obtained with TAK-044 pretreatment (10 min before occlusion). Similar reductions of the infarct size were also obtained with TAK-044 administered 1 h after reperfusion of coronary flow: a 16% reduction ($50 \pm 2\%$ of the area-at-risk, $n = 6$) at 0.3 $mg\ kg^{-1}$ and a 23% reduction ($47 \pm 3\%$ of the area-at-risk, $n = 7$) at 1 $mg\ kg^{-1}$ (Figure 5).

Discussion

The present study showed that TAK-044 is a specific endothelin receptor antagonist with a feature of antagonizing both ET_A and ET_B receptors on the basis of the following observations. The binding study showed that TAK-044 inhibited radiolabelled ET-1 binding to membrane fractions of rabbit ventricle and cerebellum. Since ET-1 inhibited the [125 I]-ET-1 binding to the ventricular preparation more than did ET-3 but both ET-1 and ET-3 showed similar inhibitory potencies on the cerebellar preparation, the major components of endothelin receptor in the ventricle appeared to be ET_A receptors and those in the cerebellum ET_B receptors (Masaki *et al.*, 1992). The binding profile of BQ-123 supported this view; it inhibited more potently in the ventricle than in the cerebellum (IC_{50} of 0.18 μ M for ET_A and 40 μ M for ET_B). In the experiments *in vivo*, transient hypotension (mainly ET_B stimulation) and a sustained hypertension (mainly ET_A

Figure 3 Effects of TAK-044 on endothelin-1-induced blood pressure changes in rats. The data are expressed as means $\pm$ s.e., $n = 5$ –9. Saline control ($\bullet$), and TAK-044: 0.01 ($\circ$), 0.1 (Δ), 1 ($\square$) and 10 ($\blacktriangle$) $mg\ kg^{-1}$. MBP: mean blood pressure.

Figure 4 Effects of TAK-044 on the extension of myocardial infarct size in rats. Columns indicate mean with s.e., $n = 12$ (Saline) and $n = 5$ –7 (TAK-044). ** $P < 0.01$, *** $P < 0.001$ compared with Saline.

Figure 5 The effects of TAK-044 administration at different times on the myocardial infarct size in rats. The three columns on the far left-hand side are from Figure 4 as the reference (Saline and Pre-occlusion). TAK-044 0.3 (darkly stippled column) and 1 mg kg⁻¹, (lightly stippled column) i.v. was administered 50 min after coronary occlusion (10 min before reperfusion started; post-occlusion (50 min)) or 1 h after reperfusion (post-reperfusion (1 h)). Columns show mean with s.e., $n = 12$ (Saline) and $n = 5-12$ (TAK-044). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with Saline.

stimulation) of rats were both inhibited by pretreatment with TAK-044, which indicates that it is an ET_A/ET_B receptor antagonist. Contractions of porcine coronary arteries evoked by various agonist, *in vitro*, were not inhibited by TAK-044, whereas those induced by ET-1, ET-2 and ET-3 were; thus TAK-044 is a selective endothelin receptor antagonist. In addition, the inhibitory effects of TAK-044 against ET-1-induced contractions of porcine coronary arteries were not endothelium-dependent.

The vasoconstriction induced by ET-3 showed two distinct phases, indicating the involvement of at least two different factors (receptors). Since ET-3 has a higher affinity for ET_B than ET_A receptors (Figure 1 and Sakurai *et al.*, 1990), the first phase of vasoconstriction (up to 10 nm in Figure 2c) is likely to be induced through stimulation of ET_B receptors

and the second-phase responses are through ET_A receptor stimulation. This suggestion is supported by the results that TAK-044 inhibited ET-3-induced vasoconstriction of porcine coronary arteries in both phases (Figure 2), while only the second phase of contraction was inhibited by BQ-123. Furthermore, although ET_B receptors have been found to cause vasorelaxation and hypotension (Masaki *et al.*, 1992), ET_B receptor-induced vasoconstriction has been demonstrated in various vessels, including canine coronary arteries (Teerlink *et al.*, 1994), swine pulmonary vein (Sudjarwo *et al.*, 1993) and rat mesenteric and renal arteries (Clozel *et al.*, 1992).

We have shown the contribution of ET-1 to the extension of infarct size in the present and the previous (Watanabe *et al.*, 1991) studies using a receptor antagonist and monoclonal antibody for ET-1, respectively. However, recent studies with ET_A antagonists provided controversial results: continuous i.v. injection of BQ-123 reduced the infarct size in dogs (Grover *et al.*, 1993), while that of FR139317 showed no effects in rabbits (McMurdo *et al.*, 1994). Since monoclonal antibody for ET-1 reduced the infarct size in rats (Watanabe *et al.*, 1991), rabbits (Kusumoto *et al.*, 1993) and dogs (unpublished observations), species-dependency is not likely to be the explanation. One of the possible explanations is, thus, organ-selectivity of the endothelin antagonist: TAK-044 was demonstrated to inhibit ET-1-induced vasoconstriction of the coronary artery more than the basilar, femoral, renal or mesenteric artery (Watanabe *et al.*, 1994). Alternatively, it is possible that ET_A/ET_B receptor antagonists show stronger inhibitory effects over ET_A receptor antagonists for limiting myocardial infarct size extension. Since increases in ET-1 play an important role in it (Watanabe *et al.*, 1991; Kusumoto *et al.*, 1993) and ET-1 has been reported to activate both ET_A and ET_B receptors (Arai *et al.*, 1990; Sakurai *et al.*, 1990), it is reasonable to assume that both receptor subtypes are involved in the extension of infarct size. The existence of ET_A and ET_B receptors in smooth muscle of artery and vein (e.g. Winkles *et al.*, 1993) and in cardiac tissues (e.g. Hori *et al.*, 1992; Molenaar *et al.*, 1993) have been demonstrated to support this view. Moreover, vasoconstriction through ET_B receptors was demonstrated in coronary arteries (Teerlink *et al.*, 1994) in addition to that through ET_A receptors. It is clear that well-designed studies focused on a role of ET_A and ET_B receptors in this model should be carried out to clarify the above issue.

We have concluded that TAK-044 is a specific ET receptor antagonist with a feature of inhibiting phenomena induced by activation of both ET_A and ET_B receptors and that it limited the extension of myocardial infarct size after coronary artery occlusion and reperfusion in rats.

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