Comparison of the acute effects of a selective endothelin \( \text{ET}_A \) and a mixed \( \text{ET}_A/\text{ET}_B \) receptor antagonist in heart failure

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Abstract

Objective: Both the selective endothelin (ET) \( \text{ET}_A \) receptor and mixed \( \text{ET}_A/\text{ET}_B \) receptor antagonists improve haemodynamics in patients and experimental models with congestive heart failure (CHF) and reduce the mortality in CHF rats. However, it remains unclear which of these antagonists is superior in the treatment of CHF. In addition, there is little information as to whether these ET receptor antagonists contribute to the neuroendocrine regulation and body fluid balance. We therefore investigated the cardiorenal and neurohumoral benefits of selective \( \text{ET}_A \) receptor and mixed \( \text{ET}_A/\text{ET}_B \) receptor antagonists in CHF.

Methods: We administered acutely either the selective \( \text{ET}_A \) receptor antagonist FR139317 (FR, \( n=6 \), 1 and 10 mg/kg) or the mixed \( \text{ET}_A/\text{ET}_B \) receptor antagonist TAK-044 (TAK, \( n=6 \), 1 and 3 mg/kg) to conscious dogs with CHF induced by rapid right ventricular pacing for ten days.

Results: Both FR and TAK decreased the cardiac pressures and the plasma atrial natriuretic peptide level and increased the cardiac output and urinary sodium excretion. FR increased the urine flow rate in association with an increased glomerular filtration rate and renal plasma flow, while TAK reduced the plasma aldosterone level. Neither antagonist increased the plasma renin activity or norepinephrine levels.

Conclusions: These findings suggest that a selective \( \text{ET}_A \) receptor antagonist has more beneficial acute effects on renal functions in CHF than a mixed \( \text{ET}_A/\text{ET}_B \) antagonist. However, the long-term administration of a mixed \( \text{ET}_A/\text{ET}_B \) receptor antagonist would improve not only the haemodynamics but also prevent fluid retention by suppressing secretion of aldosterone during the treatment of chronic CHF.

Keywords: Heart failure; Endothelin; Antagonists; Neurohumoral factors; Dog

1. Introduction

Endothelin (ET)-1, an endothelium-derived peptide [1], can bind to two ET receptors, \( \text{ET}_A \) and \( \text{ET}_B \) [2]. In vascular beds, \( \text{ET}_A \) receptors in vascular smooth muscle cells mediate vasoconstriction, while \( \text{ET}_B \) receptors mediate not only vasodilation through the release of nitric oxide and prostacyclin in endothelial cells [3] but also vasoconstriction in vascular smooth muscle [4]. Therefore, endogenous ET-1 may be involved in the complicated modulation of vascular tone through the activity of each receptor.

ET-1 can cause not only potent and prolonged vasoconstriction but also sodium retention and activation of cathecolamine, angiotensin II (AT II) and aldosterone secretion [1,5,6]. Congestive heart failure (CHF) is characterized by vasoconstriction, volume retention and the activation of such neurohumoral factors as the sympathetic nervous system and the renin–angiotensin–aldosterone system (RAAS). Therefore, ET-1 may interact with other vasoactive factors that are known to be activated in CHF and play an important role in the pathogenesis of CHF. Indeed, ET-1 circulates in the plasma, and the plasma ET-1 level has been shown to increase in patients [7,8] as well as in several animal models with CHF [9,10]. Both selective \( \text{ET}_A \) and mixed \( \text{ET}_A/\text{ET}_B \) antagonists improved not only the haemodynamics but also the survival rate in CHF rats [11,12]. However, since there is little information as to whether these ET receptor antagonists contribute to regulation of the release of neurohumoral factors and to
body fluid balance in CHF, it is still not clear which of these ET receptor antagonists has a more beneficial value for the treatment of CHF.

The identification of neuroendocrine activation has important therapeutic implications because a treatment designed to inhibit neurohumoral excitation may prevent the development of CHF. The V-HeFT II study has shown that the angiotensin converting enzyme (ACE) inhibitor, enalapril, improved mortality compared with non-specific vasodilators by suppressing the progressive activation of the RAAS and the sympathetic nervous system [13]. Therefore, for the treatment of CHF, we should evaluate the effects of the ET receptor antagonists not only on haemodynamics but also on the neurohumoral and body fluid balance, and examine the overall pathophysiological effects of endogenous ETs.

In the present study, we investigated the cardiorenal and neurohormonal effects of FR139317, a selective ET\(_A\) receptor antagonist, and TAK-044, a mixed ET\(_A\)/ET\(_B\) receptor antagonist, in conscious dogs with CHF induced by rapid right ventricular pacing, and assessed the therapeutic benefits of these ET receptor antagonists for CHF. Elucidation of ET receptor antagonists will open up a new avenue, leading to new strategies in the management of CHF.

2. Methods

This study was approved by the Animal Research Committee of Shiga University of Medical Science and conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985).

2.1. Animal preparation

Experiments were carried out in conditioned beagles of either sex (10–13 kg). All surgery to implant the cardiac pacemaker leads and intravascular catheters was performed as reported previously [14]. Briefly, the heart was exposed through a left thoracotomy incision at the fourth intercostal space. The pericardium was opened, and two cardiac unipolar pacemaker leads (M-23, Matsuda) were sutured onto the right ventricular apex. A thermodilution catheter (7 Fr.; model T-047-03, Goodtec) was advanced into the pulmonary artery via the right femoral vein for the measurement of right atrial pressure (RAP), mean pulmonary arterial pressure (MPAP), pulmonary capillary wedge pressure (PCWP) and cardiac output (CO). CO was assessed in triplicate by the thermodilution technique with 5 ml of ice-cold 0.9% saline injected into the right atrium, and the subsequent thermodilution curves were integrated by a Gould cardiac output computer (SP1435, Statham). The variation of measurement in triplicate was within 5%.

A Tygon catheter (flexible plastic tubing; Norton) was placed in the descending thoracic aorta via the right common carotid artery for measurement of the mean arterial pressure (MAP). The other Tygon tube was advanced into the vena cava via the right jugular vein for drug administration. The pacemaker (model 540, Seamed) and the ends of the catheters and pacing wires were securely fastened in a small bag worn on the back of the animal. The dogs were allowed to recover from surgery for at least 14 days before control study measurements were made. After control hemodynamic and plasma samples for neurohumoral determinations were obtained, rapid right ventricular pacing (270 beats/min) was started and continued for ten days. All subsequent studies were performed with animals in the conscious state.

2.2. Drugs

FR139317 (FR), a highly selective ET\(_A\) receptor antagonist, which has an 8868-fold higher selective affinity for the ET\(_A\) receptor than for the ET\(_B\) receptor in the porcine aorta and kidney membrane [15], was a kind present from Fujisawa Pharmaceutical Co., Ltd. (Tsukuba, Japan).

TAK-044 (TAK), which is often described as a non-selective ET receptor antagonist [16,17], was generously supplied by Takeda Chemical Industries (Osaka, Japan). Although TAK has a 244-fold higher selectivity for the ET\(_A\) receptor than for the ET\(_B\) receptor in vitro human ventricle tissue [18], TAK inhibited both ET\(_A\)- and ET\(_B\)-mediated blood pressure responses induced by exogenous ET-1 and also inhibited the S6c (ET\(_B\) agonist)-induced ET\(_B\)-mediated vasoconstriction and pressor responses in rats [16].

2.3. Experimental protocol

2.3.1. Study 1. Inhibitory effects of FR and TAK on exogenous ET-1

To confirm the in vivo effects of FR and TAK as selective ET\(_A\) and mixed ET\(_A\)/ET\(_B\) receptor antagonists, respectively, in dogs, we observed the inhibitory effects of these antagonists on the response of MAP to exogenously administered human ET-1 (Peptide Institute, Japan). After the haemodynamic status had stabilized, ET-1 (0.75 nmol/kg, \(n=4\)) was injected as a bolus, FR (1 mg/kg, \(n=4\)) or TAK (1 mg/kg, \(n=4\)) intravenously 5 min prior to ET-1 injection, and the change in MAP was recorded for 30 min in each group.

2.3.2. Study 2. Cardiorenal and hormonal effects of FR and TAK in dogs with CHF

To evaluate the cardiorenal and neurohumoral effects of selective ET\(_A\) and mixed ET\(_A\)/ET\(_B\) receptor antagonists in CHF, we divided the dogs into three groups; the FR group \((n=6)\) was given FR, the TAK group \((n=6)\) was given TAK and the vehicle group \((n=5)\) received only saline as
a control. All subsequent measurements were recorded with ongoing rapid ventricular pacing. As previously reported [14], the urinary bladder was catheterized for urine collection and creatinine (CRE) and p-aminohippurate (PAH) were infused, to calculate the glomerular filtration rate (GFR) and renal plasma flow (RPF). After a 60-min equilibration period, the first of two clearance periods was performed, each of which was 20 min in duration. After stable basal haemodynamic records were obtained, in the FR group, the first bolus dose of FR at 1 mg/kg was administered intravenously. After 30 min, the second dose of 10 mg/kg was injected, and a 30-min clearance was performed for each dose. In the TAK group, TAK was administered at 1 and 3 mg/kg at intervals of 30 min in the same way. We decided to observe the dose responsive effects using two incremental doses because a higher dose of each agent was expected to exert more favorable effects. Consequently, a three-fold higher dose (3 mg/kg) was selected. However, in the preliminary study, 3 mg/kg FR did not induce any further depressor effects on MAP compared with 1 mg/kg (by 10.5 and 10.9%, respectively, n=4, P=0.86). Therefore, we examined the administration of 1 and 10 mg/kg on the response to FR in the present study. In the vehicle group, saline was administered at intervals of 30 min, to exclude any temporal effects. During each clearance period, the cardiac pressure measurements were recorded every 5 min. Pressure data regarding MAP, RAP, MPAP and PCWP were collected. CO was determined at the end of each period. Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated as follows: 

\[
SVR; \frac{(MAP-\text{RAP})}{\text{CO}} \times 80, \quad PVR; \frac{(MPAP-\text{PCWP})}{\text{CO}} \times 80.
\]

Blood was drawn from the pulmonary artery through the indwelling thermodilution catheter at the end of each period and transferred to specially prepared tubes that were stored on ice, to analyze plasma ET-1, atrial natriuretic peptide (ANP) concentrations, plasma renin activity (PRA), plasma aldosterone and norepinephrine. Blood for analysis of plasma CRE, PAH and sodium were drawn at the midpoint of each clearance period. An equal volume of saline was injected to replace the volume lost by blood withdrawal. After the injection of antagonist, two additional 30-min urine collections were made.

2.4. Analysis of blood and urine samples

PRA and ET-1, ANP and aldosterone levels in the sampled blood were measured by radioimmunoassay (RIA) as previously described [14,19,20]. Plasma norepinephrine levels were measured by high-performance liquid chromatography. Serum and urine CRE, PAH and sodium concentrations were measured as previously reported [14]. CRE clearance and PAH clearance were calculated using standard formulas, and were equated with the GFR and RPF, respectively.

2.5. Statistical analysis

All data are presented as mean values±standard error of the mean (SE). Analysis of variance (ANOVA) for repeated measurements was used to determine the significance of changes during multiple time-dependent observations. Comparisons with the baseline values were performed by the Scheffé’s test following ANOVA for repeated measurements. The Student’s t-test was used to analyze the significance of single comparisons. Differences at a P value <0.05 were considered to be statistically significant.

3. Results

3.1. Blocking effects of FR and TAK on ET-1 injection

As shown in Fig. 1, ET-1 produced an initial transient reduction in MAP followed by a sustained increase in MAP that was maximal within 15 min of injection. Pretreatment with FR did not affect the initial hypotension, but significantly blocked ET-1-induced hypertension for...
about 30 min after ET-1 injection compared with ET-1 administration alone. On the other hand, TAK significantly inhibited both the initial transient depressor and the following pressor response to ET-1 compared with ET-1 injection alone. TAK suppressed the maximal sustained elevation in MAP (from 15 to 30 min) as much as FR.

3.2. Characteristics of CHF

The baseline haemodynamic, neurohumoral and renal functional characteristics of CHF induced by rapid ventricular pacing are summarized in Table 1. In the FR, TAK and vehicle groups, PCWP and RAP were significantly increased, but MAP and CO were decreased relative to the respective baseline values. The plasma ET-1, norepinephrine, and aldosterone levels and PRA were significantly increased to several times the baseline level. The plasma ANP concentration increased around seven-fold compared with those in the normal controls. Urine and urinary sodium excretion decreased after pacing. In addition to the deteriorated haemodynamics and activated neurohumoral secretion, all dogs were judged to be in CHF, based on evidence of anorexia and mild ascites and signs of exertional dyspnea. When the haemodynamic and neurohumoral baseline data of all of the experimental animals were pooled, the differences among the three groups were not significant.

3.3. Haemodynamic effects of FR and TAK in dogs with CHF

The haemodynamic responses to the two incremental doses of FR or TAK are shown in Fig. 2A–F. We measured the haemodynamic changes on a fixed heart rate with ongoing rapid ventricular pacing. Both FR and TAK significantly increased CO and decreased MAP and PCWP in association with significant reductions in SVR and PVR. Neither FR nor TAK caused significant changes in RAP. None of these haemodynamic variables changed significantly in the vehicle group.

Table 1
Baseline haemodynamic, neurohumoral and renal functional characteristics

<table>
<thead>
<tr>
<th></th>
<th>FR group (n=6)</th>
<th>TAK group (n=6)</th>
<th>Vehicle (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>CHF</td>
<td>Normal</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>119.0±9.0</td>
<td>100.3±0.6°</td>
<td>137.5±7.6</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>5.7±1.4</td>
<td>14.2±1.4°</td>
<td>3.2±0.8</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>2.8±0.3</td>
<td>5.2±0.4°</td>
<td>4.5±0.8</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.38±0.18</td>
<td>1.60±0.11°</td>
<td>2.43±0.08</td>
</tr>
<tr>
<td>Endothelin-1 (pg/ml)</td>
<td>2.70±0.36</td>
<td>5.65±0.67°</td>
<td>2.23±0.38</td>
</tr>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>233±29</td>
<td>584±157°</td>
<td>341±43</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>49±11</td>
<td>377±55°</td>
<td>45±4</td>
</tr>
<tr>
<td>PRA (mg/ml/h)</td>
<td>1.60±1.40</td>
<td>4.10±1.30°</td>
<td>1.40±1.60</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>32±7</td>
<td>99±18°</td>
<td>31±3</td>
</tr>
<tr>
<td>Urine flow rate (ml/min)</td>
<td>1.38±0.30</td>
<td>0.79±0.19°</td>
<td>1.18±0.20</td>
</tr>
<tr>
<td>U-Na (µM equiv./min)</td>
<td>79.8±18.3</td>
<td>11.6±5.5°</td>
<td>77.9±13.8</td>
</tr>
</tbody>
</table>

Values are expressed as means±SE.
MAP, mean arterial pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; CO, cardiac output; ANP, atrial natriuretic peptide; PRA, plasma renn activity; U-Na, urinary sodium excretion rate.
°P<0.05, °°P<0.01, °°°P<0.001 vs. normal.
terone levels. FR and TAK tended to increase the plasma norepinephrine levels, but these changes were not significant. Both FR and TAK significantly decreased the plasma ANP levels. TAK, but not FR, significantly increased the concentration of ET-1. The control saline injection did not affect these hormonal parameters.

3.5. Renal effects of FR and TAK in dogs with CHF

The effects of FR and TAK on renal functions are shown in Table 2. Only FR significantly increased the urine flow rate and RPF, while TAK affected neither. FR and TAK significantly increased GFR and the urinary sodium excretion rate compared to the average basal values.

4. Discussion

This is the first report, to our knowledge, comparing the acute effects of an ET and a mixed ET / ET receptor antagonist in CHF. Both ET receptor antagonists improved the haemodynamics and sodium retention without further activation of the sympathetic nervous system or the RAAS. While only FR increased urine output, TAK reduced the plasma aldosterone level.

4.1. Characteristics of FR and TAK in dogs in vivo

In study 1, exogenous ET-1 produced the initial depressor response, which was mediated by ET_{A} receptors via the release of relaxing factors from the vascular endothelium [3], and the subsequent pressor response, which is primarily mediated by ET_{A} receptors. Pretreatment with FR (1 mg/kg) inhibited the only sustained increase in MAP, indicating that FR is a selective ET_{A} receptor antagonist in dogs in vivo and that FR, at a dose of 1 mg/kg, exerts a sufficient ET_{A} receptor antagonistic activity. On the other hand, TAK (1 mg/kg) significantly inhibited both the ET-1-induced initial transient hypotension and the following hypertension. Although TAK has a higher selectivity for the ET_{A} receptor in vitro [16,18],

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Table 2
Renal effects of ET receptor antagonists in dogs with CHF

<table>
<thead>
<tr>
<th></th>
<th>FR group (n=6)</th>
<th>TAK group (n=6)</th>
<th>Vehicle (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 mg/kg</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>U/V (ml/min)</td>
<td>0.79±0.19</td>
<td>1.04±0.26</td>
<td>1.13±0.21*</td>
</tr>
<tr>
<td>U-Na (mEquiv./min)</td>
<td>11.6±5.5</td>
<td>17.7±7.1</td>
<td>32.3±10.9†</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>130.2±17.6</td>
<td>188.5±31.7</td>
<td>196.4±21.3†</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>34.2±3.8</td>
<td>47.7±6.4†</td>
<td>47.0±5.1†</td>
</tr>
</tbody>
</table>

Values are expressed as means±SE.

ET, endothelin; CHF, congestive heart failure; CL, clearance period; U/V, urine flow rate; U-Na, urinary sodium excretion rate; RPF, renal plasma flow; GFR, glomerular filtration rate.

*P<0.05, †P<0.01 vs. baseline.
TAK at a dose of 1 mg/kg functions as a sufficient ET_{A} and ET_{B} receptor antagonist in dogs in vivo. Moreover, FR and TAK, at doses of 1 mg/kg, almost equally suppressed the sustained elevation in MAP from 10 to 30 min, suggesting that FR and TAK may have similar ET_{A} receptor antagonistic activities at a dose of 1 mg/kg.

4.2. Changes in haemodynamics

Using a canine model of CHF, induced by intracoronary microembolization, Shimoyama et al. [10] showed that a mixed ET_{A}/ET_{B} receptor antagonist, bosentan, increased CO through an effect on SVR and, while it induced a small decrease in MAP, this change was not significant. In the present study, no haemodynamic differences were apparent between the selective and mixed ET receptor antagonists, indicating that overall endogenous ETs act predominantly as vasoconstrictors (via ET_{A} and constrictive ET_{B} receptors) and play an important role in the maintenance of the haemodynamics in CHF. However, we recently demonstrated that a selective ET_{B} antagonist, RES-701-1, increased MAP, SVR and PVR and reduced CO in dogs with CHF [19]. Therefore, vasodilative ET_{B} receptors on the endothelium greatly contribute to the regulation of blood pressure in CHF, and the blockade of vasodilative ET_{B} receptors may be harmful in the treatment of CHF. We suppose that a selective ET_{B} receptor antagonist may have the advantage of maintaining ET_{B} receptor-mediated vasodilatation in the treatment of CHF. If possible, the specific antagonism of both ET_{A} and constrictive ET_{B} receptors may be the most beneficial.

4.3. Changes in plasma hormones

The identification of neurohumoral alternations is important because the treatment of CHF should be designed to inhibit vasoconstrictive neuroendocrine excitation as ventricular dysfunction progresses and develops into CHF.

In theory, with a fall in blood pressure, the sympathetic nervous system can be stimulated [21]. However, neither FR nor TAK caused a significant increase in the plasma norepinephrine levels in this study. Both FR and TAK may have the potential to suppress the release of norepinephrine, because an ET_{A} receptor antagonist, BQ-123, inhibited ET-1-induced norepinephrine secretion in the canine adrenal gland in vivo [22].

In the present study, neither FR nor TAK caused a significant change in PRA. Although ET-1 has been demonstrated to have an inhibitory effect on renin release in vitro [23], there have been conflicting findings in vivo where ET-1 both decreased and increased PRA in dogs with CHF [6,24]. However, we do not believe that ET-1 is primarily concerned with the release of renin. The macula densa mechanism and renal baroreflex are major stimuli for renin release [25]. The increased urinary sodium excretion by both FR and TAK may suppress renin release. As the arterial blood pressure was decreased by FR and TAK, the renal perfusion pressure might also be decreased and, consequently, the reduced renal perfusion pressure might stimulate renin release. These modulators of renin release might counteract each other and, thereby, the PRA did not significantly change after administration of FR and TAK.

However, TAK, but not FR, significantly suppressed the concentration of plasma aldosterone, despite no significant decline in PRA. Neither TAK nor FR altered the plasma AT-II and potassium levels throughout the experiment (data not shown). As for the secretion of aldosterone, ET-1 may be significantly involved through ET_{B} receptors, but not via the renin angiotensin axis or plasma potassium level. Indeed, a specific ET_{A}, receptor antagonist, BQ-788, decreased the ET-1-stimulated output of aldosterone by zona glomerulosa cells in rats [26]. Moreover, we recently demonstrated that a selective ET_{B} antagonist, RES-701-1, reduced the plasma aldosterone level in the same canine model of CHF [19]. For the long-term treatment of CHF, the chronic administration of a mixed ET_{A}/ET_{B} receptor antagonist would prevent volume retention by suppressing the secretion of aldosterone.

We have previously reported that endogenous ANP inhibited the secretion of ET-1 through a cGMP pathway in dogs with CHF [22], while ET-1 stimulates to release ANP [5,27]. It is important to clarify the relationship between ET-1 and ANP because both peptides counteract each other in CHF. An ET_{A} receptor antagonist, BQ-123, inhibited ET-stimulated ANP secretion in rat atrial myocytes and an ET_{B} antagonist, RES-701-1, did not affect the plasma ANP level in dogs with CHF [19]. Endogenous ET may mediate ANP secretion through the ET_{A} receptor in dogs with CHF. However, we cannot exclude the possibility that the reduction in ANP with FR and TAK may reflect haemodynamic improvement expressed as a lowering PCWP. In any case, the decrease in ANP levels with both FR and TAK may suggest that antagonism with ET_{A} receptors, but not ET_{B} receptors, can attenuate the exacerbation of CHF because ANP is secreted in proportion to severity of CHF and is a marker for the prognosis of patients with CHF [28].

FR did not affect ET-1 concentrations in the plasma. In contrast, TAK significantly increased the plasma ET-1 levels in a dose-related manner. We and other investigators have demonstrated that the selective blockade of ET_{B} receptors, but not of ET_{A} receptors, increases the plasma ET-1 level [10,12,19], suggesting that ET_{B} receptors appear to mediate the clearance of ET-1. Changes in the circulating ET-1 concentrations would be useful to show a significant difference between these two ET receptor antagonists.

4.4. Changes in renal functions

ET-1 enhances water conservation mainly via ET_{A}
receptors, by glomerular vasoconstriction [29]. In contrast, ET-1 impedes water conservation via ET$_B$ receptors by blunting arginine vasopressin-mediated water reabsorption [29,30] and by producing prostaglandin E2 [30] in rats. In addition, ET$_A$ receptor activation results in diuresis, but does not alter sodium excretion in dogs [31]. A selective ET$_A$ antagonist (FR) might attenuate renal vascular contraction and increase RPF and GFR. Thus, FR induced the increase in urine and sodium excretion due to the increased GFR and RPF. On the other hand, a selective ET$_B$ antagonist reduced RPF and tended to decrease GFR and urinary sodium and water excretion in dogs with CHF [19]. Therefore, it is possible that TAK, a mixed ET$_A$ and ET$_B$ receptor antagonist, did not alter urine output or RPF and caused only a small increase in GFR and urinary sodium excretion by cancelling one effect mediated by the ET$_A$ receptor and the other mediated by the ET$_B$ receptor. The roles of endogenous ETs in renal sodium and water handling mediated by ET$_A$ and ET$_B$ receptors seem to be very complex, and further investigation is required.

Only FR has a beneficial acute diuretic effect, suggesting that the acute administration of a selective ET$_A$ receptor antagonist is more advantageous to renal functions than that of a mixed ET$_A$/ET$_B$ antagonist. However, the CONSENSUS I trial [32] and the SOLVD trial [33] have shown that ACE inhibitors, which have little effect on diuresis in CHF [34], improve mortality and morbidity in chronic CHF, indicating that management of neurohumoral factors, but not of urination, is the most important factor in the chronic treatment of CHF. Since the characteristics of ET receptor antagonists are fairly similar to those of ACE inhibitors, both selective ET$_A$ and mixed ET$_A$/ET$_B$ receptor antagonists have great possibilities as therapeutic agents for chronic CHF.

### 4.5. Study limitations

It is possible that the selective ET$_A$ receptor antagonist’s (FR) blocking effects mediated by the ET$_A$ receptors might simultaneously augment the effects mediated by the ET$_B$ receptors. In addition, although TAK had an antagonistic activity against both vasodilative and constrictive ET$_B$ receptors as well as ET$_A$ receptors, it remains unclear how much of each ET$_B$ receptor (vasodilative and constrictive) is concerned with the regulation of blood pressure and we could not quantitatively distinguish the haemodynamic effect mediated by these ET receptors (ET$_A$ receptor and both ET$_B$ receptors) on one another.

We could not also exclude the possibility of interaction between haemodynamic and hormonal effects because of systemic administration of antagonists. Although the heart rate was fixed with ongoing rapid pacing, the vascular responses to any vasoconstrictor or drug may be modified by cardiovascular reflex responses, including arterial and cardiopulmonary baroreflexes or changes in the circulating levels of hormones that affect the cardiovascular system.

### 4.6. Conclusions

Without activation of the sympathetic nervous system and the RAAS, despite a reduction in blood pressure, both FR and TAK improved the haemodynamics, significantly reduced the plasma ANP level and increased the excretion of sodium in dogs with CHF. While only FR increased the urine output, TAK reduced the plasma aldosterone level. These findings suggest that a selective ET$_A$ receptor antagonist has more beneficial acute effects on renal functions than a mixed ET$_A$/ET$_B$ antagonist. However, as CHF is a chronic disease, assessment of long-term treatment with those agents is required. The chronic administration of a mixed ET$_A$/ET$_B$ receptor antagonist may prevent volume retention by suppressing the secretion of aldosterone. Our complementary results would be helpful to understand the pathophysiological roles of ET-1 and have important therapeutic implications.

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### References


