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# Effects of endothelin receptor A antagonist FR139317 on rats with congestive heart failure[1]

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**KEY WORDS** congestive heart failure; rats; endothelin receptors; FR139317; hemodynamics; mortality; endothelin-1

## ABSTRACT

**AIM**: To study the effects of a selective endothelin receptor A (ETA) antagonist FR139317 on rats with congestive heart failure. **METHODS**: A congestive heart failure model was established via left coronary artery ligation in adult male Wistar rats. The rats with congestive heart failure were treated with FR139317 at two doses (1 and 5 $mg \cdot kg^{-1} \cdot d^{-1}$ respectively for 6 weeks) or with vehicle. Hemodynamics, plasma level of endothelin-1 (ET-1), and mortality rate of rats were measured. **RESULTS**: Both groups treated with FR139317 (high and low dose) have lower mortality rate (25.0 % and 28.6 % *vs* 50.0 %) and lower plasma level of ET-1 than that of vehicle [(3.6 ± 1.2) ng/L and (4.9 ± 1.5) ng/L *vs* (5.8 ± 1.3) ng/L]. Comparing to vehicle group, left ventricular end-diastolic pressures of the FR139317-treated groups were improved significantly [(12 ± 6) mmHg and (14 ± 7) mmHg *vs* (22 ± 9) mmHg]. FR139317 at a higher dose reduced the mean arterial pressure of the rats with congestive heart failure and decreased the plasma concentration of endothelin to a closer level of rats with normal heart function than lower dose. **CONCLUSION**: Selective ETA antagonist FR139317 improved the hemodynamics and reduced the plasma ET-1 level and the mortality of rats with congestive heart failure.

## INTRODUCTION

It is well documented that activation of neurohumoral factors from sympathetic nervous system or rennin-angiotensin-aldosterone system plays an important role in the pathogenesis of congestive heart failure (CHF). Indeed, another neurohumoral factor, endothelin-1 (ET-1), has been shown to be activated in patients as well as in animal models with CHF[1]. The underlying mechanism of ET-1 in the progression of CHF is complex, in addition to its vasoconstrictor effects, ET-1 has been shown to augment the inotropic function of the heart, stimulate cardiomyocyte hypertrophy, promote collagen turnover in cardiac fibroblast, *etc*[2]. Acute administration of drugs blocking the generation or actions of endogenous ET-1 exerts favorable hemodynamic effects on CHF in animal and human subjects[3,4]. Encouragingly, chronic treatment with either an endothelin receptor subtype A (ETA) selective or subtype A and subtype B (ETB) dual antagonist substantially reduced mortality in animal models of CHF[5,6]. However, on the other hand, both selective ETA and dual ETA/ETB antagonists are potent vasodilators, and the ensuing hypotension of an overdose may diminish or eliminate the favorable effect of anti-endothelin therapy, as was shown in the CONSENSUS II study engaging enalapril inhibiting the activity of angiotensin-converting-enzyme[7].

FR139317 is a potent ETA selective antagonist. It was reported that FR139317 inhibited the specific binding of [$^{125}$I]endothelin-1 to porcine aortic microsomes in a concentration-dependent fashion with an $IC_{50}$ of 0.53 nmol/L *in vitro*[8]. Ohnishi *et al*[9] proved that a single intravenous dose of FR139317 1 $mg \cdot kg^{-1}$ revealed a 10.9 % of depressor effect on mean arterial pressure, when the dose increased to 3 $mg \cdot kg^{-1}$ the mean arterial pressure was not depressed further more, while at the dose of 10 $mg \cdot kg^{-1}$ FR139317 decreased the arterial pressure significantly in an acute experiment in dogs with CHF.

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This study was undertaken to determine the effect of long-term therapy with two different doses of selective ETA antagonist FR139317 on the hemodynamics, plasma ET-1 level, and mortality in rats with CHF following myocardial infarction.

## MATERIALS AND METHODS

**Rats** Male adult Wistar rats (Grade Ⅱ, Certificate № 99A004) weighing 250 – 300 g were obtained from the Experimental Animal Center of Nanfang Hostpital (Guangzhou, China). All rats were maintained on standard rat feed and water *ad libitum*.

**Reagents** FR139317 was a product of Fujisawa Pharmaceutical Co, Ltd (Japan). ET-1 RIA kit was perchased from the General Hospital of PLA (Beijing, China).

**Preparation of congestive heart failure model** Left coronary artery ligation was performed in adult male Wistar rats as previously described[10]. Briefly, each rat was anesthetized with pentobarbital (30 $mg \cdot kg^{-1}$, ip), intubated, and ventilated with a rodent respirator. A left thoracotomy was performed, the heart exteriorized and a ligature placed around the proximal left coronary artery. Subsequently, the heart was returned to its normal position and the thorax was closed. The mortality rate of this procedure was about 40 % within the first 24 h following the operation.

**Therapeutic protocol** Two doses of FR139317, 1 $mg \cdot kg^{-1} \cdot d^{-1}$ (low-dose) and 5 $mg \cdot kg^{-1} \cdot d^{-1}$ (high-dose) were adopted in the present study. FR139317 was prepared in solutions at concentrations of 1 and 5 g/L for injection respectively. The drug was given subcutaneously from the 7th postoperative day when the rats stabilized from acute myocardial infarction and the treatment lasted for 6 weeks. Sixty surviving rats were randomized into three groups ($n$ = 20 in each group) receiving two different doses of the drug or vehicle (CHF control group). Seven rats with normal heart function received sham-operation without any treatment served as normal heart function control.

**Hemodynamic measurements** Hemodynamic measurements were performed 24 h after the last administration of the drug at the end of therapy. After anesthetized with pentobarbital, the right carotid artery and jugular vein were cannulated with polyethylene cannulas. Pressures were measured through a segment of fluid-filled $PE_{50}$ tube connected to the pressure monitor system (Model Transcope 12, Marquette Co, USA). The carotid cannula was briefly advanced to the left ventricle to measure left ventricular end-diastolic pressures (LVEDP), then withdrawn to the aorta for continuous measurement of mean arterial pressure (MAP). The jugular cannula was advanced to the right atrium for the measurement of mean right atrium pressure (MRAP). Hemodynamic measurement was carried out over a 10-min period to establish a stable state. All the hemodynamic parameters including heart rate (HR) were monitored under spontaneous respiration and the whole procedure was completed in 20 min.

**ET-1 assay** After hemodynamic measurement, 5 mL of blood samples drawn from carotid artery were collected into tubes containing edetic acid and aprotinin and kept on ice until centrifugating at 1500 × $g$ and 4 ℃ for 10 min. The supernatants were stored at – 20 ℃ before analysis. Plasma level of ET-1 was measured with commercially available radioimmunoassays according to the manufacture's instructions. Briefly, rabbit ET-1 antiserum was incubated with standard or sample for 24 h at 4 ℃; [$^{125}$I]ET-1 was added for a subsequent 24-h of incubation at 4 ℃. Bound counts were separated by precipitation at 25 ℃ for 20 min. After centrifugation at 1800 × $g$ for 25 min, the free fraction was aspirated and the pellets were counted in a gamma counter. A standard curve was constructed by dilution of synthetic ET-1. The intra-assay and iner-assay variability is < 10 % and < 15 %, respectively.

**Determination of infarct size** The infarct size of myocardium was calculated according to the technique described by Hackel *et al*[11]. The hearts were rinsed to rid remaining blood, and four evenly spaced transverse cuts were then made from the base to the apex of the ventricles dividing them into five segments of equal thickness. Then the gross involvement by infarct of each segment were recorded in their correponding cross-section diagrams, in which the segments were divided into six pieces: the free wall of the left ventricle was divided in thirds into anterior, lateral, and posterior; the septum was divided in half into anteroseptal and posteroseptal; and right ventricular free wall. According to Hackel's study, each piece accounts for a definite percentage of the total heart weight or left ventricular weight. Infarct size was then estimated as the sum percentage of all pieces involving infarct and was expressed as the percentage of left ventricle weight.

**Excluding criteria** There was a linear correlation between the infarct size and LVEDP ($r$ = 0.98, $P$ < 0.01) confirmed via linear regression study in the CHF

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control group. The regression formula was LVEDP = 2.0 + 36.9 × IS (infarct size). LVEDP≥15 mmHg was accepted as a standard of CHF in the present study. According to the formula it could be derived that the rats with infarct size equal to or more than 35.2 % of left ventricle weight would develop CHF. The rats with infarct size less than 35.2 % were excluded in the present study. The numbers of rat remained was 14 in CHF control group, 14 in the low-dose group, and 12 in the high-dose group, respectively.

**Statistical analysis** All data except mortality rate were expressed as $\bar{x} \pm s$. Linear regression was performed to study the correlation between infarct size and LVEDP in the CHF control group. Comparison of mortality rate was analyzed by a Chi-square method. One-way analysis of variances was performed to determine the significance of the difference among various groups. Differences were considered significant at the level of $P < 0.05$.

## RESULTS

**Mortality** At the end of 7th postoperative week, the mortality rate of CHF control group was 50.0 %, while that of the low-dose group was 28.6 % and the high-dose group was 25.0 %. All rats in sham-operated group were survived. The mortality rate of two groups treated with FR139317 was significantly lower than that of CHF control group. There was no significant difference between the two treatment groups (Fig 1).

**Characteristics of CHF** The ratios of heart weight and lung weight to body weight in CHF control group were significantly higher than those of sham-operated group ($P < 0.05$, Tab 1). The heart rate in the CHF control group increased significantly compared with that of sham-operated group, while the mean arterial

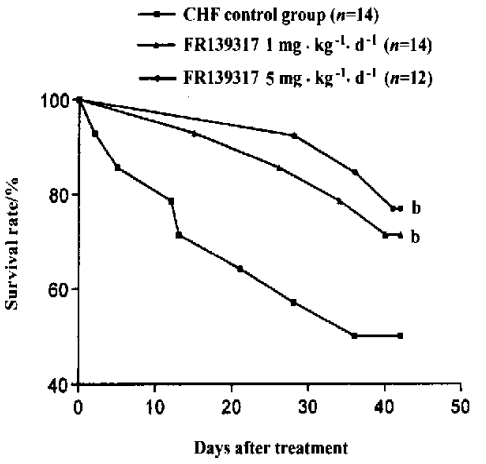


**Fig 1. The survival curve of rats with CHF. $^bP < 0.05$ *vs* CHF control group.**

pressure decreased significantly ($P < 0.05$, Tab 2). Left ventricular end-diastolic pressure and mean right atrium pressure of the CHF control groups were higher than those of sham-operated group. Plasma ET-1 level was markedly enhanced in rats of the CHF control group as compared to sham-operated group [(5.8 ± 1.3) ng/L *vs* (2.3 ± 0.8) ng/L, $P < 0.05$, Tab 2].

**Effects of FR 139317 on rats with CHF** There were no significant difference among the infarct size of the three CHF groups. As compared to those of the CHF control group, the ratios of heart weight to body weight (HW/BW) of two different doses of FR139317 treated groups were significantly decreased ($P < 0.05$), as well as the ratios of lung weight to body weight (LW/BW) (Tab 1). After 6 weeks of treatment, FR139317 at a high or low dose significantly decreased the heart rate of rats with CHF compared to that of CHF control. FR139317 at either higher or lower dose significantly decreased LVEDP, MRAP, and ET-1 level ($P < 0.05$)

**Tab 1. The infarct size, body weight, and ratios of heart and lung weight to body weight. $\bar{x} \pm s$. $^bP < 0.05$ *vs* sham-operated group. $^eP < 0.05$ *vs* CHF control group.**

| Group | *n* | Infarct size (% of LV weight) | BW (g) | HW/BW (mg/g) | LW/BW (mg/g) |
|---|---|---|---|---|---|
| Sham-operated | 7 | – | 256 ± 51 | 3.1 ± 0.5 | 6.4 ± 1.2 |
| CHF control | 7 | 39 ± 12 | 240 ± 68 | 4.1 ± 0.9$^b$ | 8.5 ± 1.4$^b$ |
| CHF + LD | 10 | 41 ± 14 | 230 ± 48 | 3.4 ± 0.8$^{be}$ | 7.5 ± 1.3$^{be}$ |
| CHF + HD | 9 | 41 ± 14 | 237 ± 44 | 3.6 ± 0.9$^{be}$ | 7.1 ± 1.8$^{be}$ |

LV: left ventricle; BW: body weight; HW: heart weight; LW: lung weight; CHF + LD: CHF treated with FR139317 1 mg·kg$^{-1}$·d$^{-1}$; CHF + HD: CHF treated with FR139317 5 mg·kg$^{-1}$·d$^{-1}$.

Tab 2. Effects of FR139317 on hemodynamic parameters and plasma ET-1 level. $\bar{x} \pm s$. $^bP < 0.05$ *vs* sham-operated group. $^cP < 0.05$ *vs* CHF control group. $^hP < 0.05$ *vs* CHF + LD group.

| Group | *n* | HR/bpm | MAP/mmHg | LVEDP/mmHg | MRAP/mmHg | ET-1/ng·L$^{-1}$ |
|---|---|---|---|---|---|---|
| Sham-operated | 7 | 374 ± 21 | 114 ± 11 | 3.4 ± 1.5 | 2.6 ± 1.0 | 2.3 ± 0.8 |
| CHF control | 7 | 399 ± 18$^b$ | 102 ± 10$^b$ | 22 ± 9$^b$ | 4.5 ± 1.6$^b$ | 5.8 ± 1.3$^b$ |
| CHF + LD | 10 | 380 ± 16$^c$ | 106 ± 16$^b$ | 14 ± 7$^{bc}$ | 3.1 ± 1.4$^c$ | 4.9 ± 1.5$^{bc}$ |
| CHF + HD | 9 | 377 ± 15$^c$ | 93 ± 12$^{bch}$ | 12 ± 6$^{bc}$ | 3.0 ± 1.4$^c$ | 3.6 ± 1.2$^{bch}$ |

HR: heart rate; MAP: mean artery pressure; LVEDP: left ventricular end-diastolic pressure; MRAP: mean right atrium pressure; CHF + LD: CHF treated with FR139317 1 mg·kg$^{-1}$·d$^{-1}$; CHF + HD: CHF treated with FR139317 5 mg·kg$^{-1}$·d$^{-1}$.

compared with CHF control group (Tab 2).

**Comparison between the effects of two doses of FR139317** No significant difference was observed in terms of body weight, ratios of heart weight and lung weight to body weight (Tab 1), as well as heart rate, LVEDP, and MRAP (Tab 2) between high dose and low dose of FR139317 treatment. However, after 6 weeks of treatment, high-dose of FR139317 markedly decreased the MAP of rats with CHF, while low-dose did not result in significant changes. Either dose of FR139317 decreased the plasma ET-1 level, but the higher dose decreased it to a level closer to that of normal heart function (Tab 2).

## DISCUSSION

The present study demonstrated that 6-week therapy with a selective ETA antagonist FR139317 prevented rats against progression of CHF following myocardial infarction. The benefits of FR139317 were shown by 1) improvement of hemodynamics; 2) attenuation of the pulmonary congestion suggested by reduced ratio of lung weight to body weight; 3) decrease in plasma level of ET-1; 4) reduction of mortality rate. The reduced ratio of heart weight to body weight implied that FR139317 attenuated left ventricular remodeling confirmed widely in previous studies[5,12].

In theory, an optimal treatment against neurohumoral excitation in CHF should reach a maximal inhibition with the best tolerability. Our data showed that FR139317 at dose of 5 mg·kg$^{-1}$·d$^{-1}$ reduced plasma ET-1 in rats with CHF to a level closer to that of normal heart function rats comparing to the low-dose (1 mg·kg$^{-1}$·d$^{-1}$) group, and decreased the MAP of rats with CHF significantly. It is proved that ETA antagonist played an important role in regulation of arterial compliance and peripheral vascular resistance in either normal or heart failure conditions. An appropriate decrease of blood pressure might be benefit for decrease afterload of the failing heart, but on the other side, a resultant hypotension of overdose can result in inadequate perfusion of vital organs including the heart itself, thus deteriorate the hemodynamic disorder of CHF. Our data is not adequate enough to provide more evidence to figure out an optimal dose of FR139317 in the treatment of CHF. Nevertheless, it can be supposed that with a tolerant blood pressure, the more reduction of plasma ET-1 to a normal level, the better prognosis of congestive heart failure will be.

Influences of ETA antagonist on plasma level of ET-1 were reported differently in some studies. Spieker *et al*[13] reported recently that after 2 h of administration, a selective ETA antagonist LU135252 increased the plasma ET-1 at a dose-dependent fashion. In the present study, ET-1 was reduced after a 6-week therapy of FR139317. One possible explanation of the conflicting results between an acute experiment and our chronic study is that the acute blockade of ETA displaces ET-1 from its receptors and induces a temporary peak in plasma, while the chronic blockade of ETA inhibits the activation of the ET-1 followed by a subsequent interaction with other neurohumoral factors. As the progression of CHF has been prevented, the production of ET-1 reduced. On the other hand, the blockade of combination of ET-1 to ETA receptors may enhance it to combine with ETB receptors contributing to clearance of ET-1.

Additionally, plasma level of ET-1 remained unchanged in some studies involving chronic treatment of ETA antagonist in CHF animal models[5,12]. In the present study, we detected the plasma ET-1 of arterial blood instead of venous blood adopted in other reports. Recently, Kjekshus *et al*[14] proved that the differences of ET-1 between the artery and vein was more than 70 % of the venous value, and ET-1 gradient over the

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cardiopulmonary circulation (aortic minus right atrial values) increased from (−0.1 ± 0.3) ng/L in normal pigs to (4.5 ± 0.6) ng/L in CHF ones and correlated strongly with arterial ET-1 level. The difference of the ET-1 levels between vein and artery in CHF subjects implies that the arterial ET-1 level is more sensitive to reflect the alteration of ET-1 in cardiopulmonary circulation. Furthermore, other factors including species difference, degree of heart failure or methodological aspects such as varying cross-reactivity toward big ET, could cause different results as well.

In conclusion, the present study demonstrated that FR139317 treatment had beneficial effects on hemodynamics and survival rate in rats with congestive heart failure after large infarcts. Because an higher dose of FR139317 showed more reduction of plasma ET-1 level and a significantly decrease in arterial blood pressure, establishment of an optimal dose could be of great importance via large and long investigation with more gradients in choice of dose.

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## 内皮素受体 A 拮抗剂 FR139317 对充血性心力衰竭大鼠的作用[1]

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**关键词** 充血性心力衰竭; 大鼠; 内皮素受体; FR139317; 血液动力学; 死亡率; 内皮素-1

**目的**: 研究不同剂量内皮素受体 A 拮抗剂 FR139317 对大鼠心力衰竭模型的作用. **方法**: 结扎雄性 Wistar 大鼠左冠状动脉致心肌梗死并充血性心力衰竭, 给予 FR139317 1 和 5 $mg \cdot kg^{-1} \cdot d^{-1}$ 两种剂量治疗 6 周, 观察内皮素受体 A 拮抗剂对心衰大鼠血液动力学、血浆内皮素水平及死亡率的作用, 并比较不同剂量间的差异. **结果**: 二种不同剂量 FR139317 治疗均改善心衰大鼠的血液动力学, 降低血浆内皮素水平, 并降低死亡率. 较高剂量 FR139317 明显降低心衰大鼠的平均动脉压, 并使血浆内皮素的浓度降至更接近于心功能正常大鼠的水平. **结论**: 选择性内皮素受体 A 拮抗剂 FR139317 可有效地治疗心力衰竭.

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