Journal of the American College of Cardiology
© 2002 by the American College of Cardiology
Published by Elsevier Science Inc.

Vol. 39, No. 1, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(01)01692-8

EXPERIMENTAL STUDIES

Short-Term Endothelin Receptor Blockade With Tezosentan Has Both Immediate and Long-Term Beneficial Effects in Rats With Myocardial Infarction

Martine Clozel, MD,* Changbin Qiu, MD, PHD,*† Chang-Shen Qiu,*† Patrick Hess,* Jean-Paul Clozel, MD*

Allschwil, Switzerland; and Shanghai, China

OBJECTIVES We investigated the effects of short-term tezosentan treatment on cardiac function, pulmonary edema and long-term evolution of heart failure (HF) in a rat model of myocardial infarction (MI).

BACKGROUND Endothelin (ET) may play a major role in the progression from MI to HF. Tezosentan is a new dual ET_A/ET_B receptor antagonist.

METHODS Rats were subjected to coronary artery ligation and were treated with either vehicle or tezosentan (10 mg/kg IV bolus) at 1 h and 24 h after MI. Cardiac hemodynamics and lung weight were measured at 48 h after MI. Survival was assessed over a five-month period.

RESULTS At 48 h after ligation, vehicle-treated rats developed HF, as evidenced by a marked increase in left ventricular end-diastolic pressure (LVEDP), reduction in dP/dt_{max} and mean arterial pressure (MAP), and development of pulmonary edema. Tezosentan treatment attenuated the increase in LVEDP and in lung weight and slightly reduced MAP without affecting dP/dt_{max}. Infarct size was not modified by tezosentan. Despite the fact that treatment with tezosentan was stopped after 24 h, the initial tezosentan administration significantly reduced cardiac hypertrophy (22%) and decreased mortality by 51% at five months (50% survival vs. 19% survival in vehicle-treated rats, $p < 0.001$).

CONCLUSIONS Tezosentan administered during the first day after MI in rats, in addition to improving acutely hemodynamic conditions, markedly increases long-term survival. This increase is associated with a decrease of pulmonary edema and prevention of cardiac hypertrophy. Tezosentan could be a safe and useful therapeutic agent in the prevention and treatment of ischemic HF. (J Am Coll Cardiol 2002;39:142–7) © 2002 by the American College of Cardiology

Endothelin (ET) plays a major role in the pathophysiology of chronic heart failure (CHF) (1,2). Significant elevations of plasma big ET-1 and ET-1 levels are observed in animal models and patients with CHF, and these elevations are strongly related to survival in patients with CHF (1–8). The transition from left ventricular (LV) dysfunction to overt heart failure (HF) can be prevented by long-term treatment with the dual ET receptor antagonist bosentan. Long-term treatment with bosentan has a beneficial effect on hemodynamics in animal models and in patients with CHF (3–5,9–12). Long-term treatment with ET receptor antagonists greatly improves the survival of rats with CHF (9,10).

Endothelin may play also an important role in the consequence of myocardial infarction (MI). Endothelin-1 levels are markedly elevated in patients with MI or unstable angina (13), and plasma concentrations of ET-1 are the best predictive factor of one-year mortality after MI (8). Finally, long-term treatment with bosentan prevents the development of cardiac hypertrophy and ventricular dilation when started early (14,15) or late (9) after MI induction in rats. However, little is known on the effects of ET receptor antagonists in the acute phase (first 2 days) after MI and whether a short-term (<2 days) ET receptor blockade could have a long-lasting beneficial effect.

Tezosentan is a new, potent dual ET_A/ET_B receptor antagonist designed for parenteral use (16). It is currently in advanced clinical development for the treatment of acute HF. The present study was designed to investigate in a rat model whether acute ET receptor blockade with tezosentan has short-term and long-term beneficial effects after MI. For this purpose, the effects of tezosentan on cardiac function and pulmonary edema were evaluated two days after MI. Then survival was evaluated during the five months after MI while treatment with tezosentan was given only for 24 h after MI.

The present study shows that ET receptor blockade can have long-lasting beneficial effects after MI, even when given only during the first day after MI.

MATERIALS AND METHODS

Studies were performed on 218 male normotensive Wistar rats weighing 200 to ~270 g. All rats were housed in

From *Actelion Ltd., Allschwil, Switzerland; and the †Department of Pharmacology, Shanghai Institute of Materia Medica, Shanghai, China. This work was supported by Actelion Ltd., Allschwil, Switzerland.

Manuscript received February 22, 2001; revised manuscript received September 10, 2001, accepted September 10, 2001.

Abbreviations and Acronyms

BW	= body weight
CHF	= chronic heart failure
ET	= endothelin
HF	= heart failure
HR	= heart rate
LV	= left ventricle
$LVdP/dt_{max+}$	= maximal rate of positive rise of left ventricular pressure
LVEDP	= left ventricle end-diastolic pressure
MAP	= mean arterial pressure
MI	= myocardial infarction
RV	= right ventricle, right ventricular

climate-controlled conditions with a 12-h light/dark cycle and free access to normal rat chow and drinking water. The animals were handled according to the "Position of the American Heart Association on Research Animal Use" adopted November 11, 1984, by the American Heart Association.

Induction of MI. Myocardial infarction was produced using a previously described method (9). In brief, rats were anesthetized with a mixture of ketamine-Rompun (50 mg/kg to 5 mg/kg, IP). The trachea was intubated (20G cannula), and the lungs were ventilated with a rodent ventilator (Model 7025 Rodent Ventilator, Hugo Sachs Elektronik, March-Hugstetten, Germany) at a rate of 60 cycles/min and a tidal volume of 1 ml/100 g body weight (BW). A left thoracotomy was performed, and the left coronary artery was ligated approximately 2 mm from its origin with a 6-0 silk suture, between the pulmonary artery outflow tract and left atrium. The sham-operated rats were subjected to the same procedure, except that the coronary artery was not ligated. The chest was then closed in three layers (ribs, muscles and skin). The remaining air was aspirated from the thorax, allowing the rats to resume spontaneous respiration. The rats were allowed to recover from anesthesia and returned to their cages.

Evaluation of the effects of tezosentan. Tezosentan (Ro 61-0612, 10 mg/kg, IV) or vehicle (saline, 1 ml/kg, IV) was administered at 1 h and 24 h after MI. Sham-operated rats were treated with saline at the same time points. Different sets of experiments were then performed.

CARDIAC HEMODYNAMICS AT 48 H. Cardiac hemodynamics was assessed at 48 h after coronary ligation or sham operation. Rats subjected to coronary artery ligation were treated with tezosentan (n = 11) or vehicle (n = 10). The third group consisted of sham-operated rats (n = 7). At 48 h after MI, rats were anesthetized with sodium hexobarbital (Inactin, 100 mg/kg, IP). Body temperature was maintained at 36 to 38°C, and a tracheotomy was performed. A 2F high-fidelity catheter (SPR-249, Millar Instruments Inc., Houston, Texas) was inserted via the right carotid artery into the left ventricle (LV) for recording of LV pressures, heart rate (HR) and maximal rate of rise of LV pressure (dP/dt_{max}). A polyethylene catheter was placed into the left femoral artery and connected to a pressure transducer (MLT1050 precision BP transducer, AD Instruments, Hastings, United Kingdom) for continuous blood pressure measurement. The data acquisition system consisted of a PowerLab (ML780 PowerLab/8s and ML118 QUAD amplifiers, AD Instruments, Hastings, United Kingdom) connected to a HP Pavilion 8565C computer with Chart software (version 3.4, AD Instruments, Hastings, United Kingdom).

After surgery and a stabilization period of 15 min, mean arterial pressure (MAP), LV systolic pressure, LV end-diastolic pressure (LVEDP), HR and dP/dt_{max} were recorded for 30 min. Calibration of the Millar catheter was verified before and after each measurement.

LUNG WEIGHT AND MORPHOLOGY. In another set of experiments, lung weight was determined in the same three groups of animals: MI rats treated with tezosentan (n = 23) or vehicle (n = 24) and sham-operated rats (n = 10). The rats were sacrificed 48 h after coronary artery ligation or sham operation, and the lungs were removed and weighed. The lungs were fixed in 10% buffered formalin and embedded in paraffin. Sections of 4 μm thickness were stained with hematoxillin and eosin and examined under light microscopy. In this set of experiments, morphometric evaluation of the LV was performed to determine the infarct size, as described below.

LONG-TERM SURVIVAL STUDY. In a third study, infarct size, cardiac weight and survival rate were analyzed after MI and treatment with tezosentan or saline. Three groups of rats, tezosentan-treated rats (n = 54), vehicle-treated rats (n = 59) and sham-operated rats (n = 20), were studied over 150 days. Animals were inspected for death twice a day on weekdays and daily on weekends and holidays. For each rat dying beyond seven days after MI and during the five-month period, BW was measured, and the heart was dissected and weighed. The right ventricle (RV) and LV plus septum (LV+S) were separated and weighed. Morphometric evaluation of the LV was performed to determine the infarct size. The animals surviving at 150 days after coronary ligation were sacrificed and similar measurements were performed. Body and heart weights (total heart, RV, LV+S) were measured, and the LV was processed for morphometric evaluation of infarct size.

MORPHOMETRIC EVALUATION OF INFARCT SIZE. After dissection, the LV+S was fixed in 10% buffered formalin for morphometric evaluation of infarct size. The LV was cut from apex to base into four transverse segments. The middle two segments, representing the bulk of the LV, were embedded in paraffin, sectioned and stained. Four slices from these segments were projected onto a screen for morphometry. The entire length of the endocardial circumference and the segment of the endocardial circumference represented by the infarcted segment of each of the four

Table 1. Cardiac Hemodynamics at 48 h After MI or Sham Operation

	MAP (mm Hg)	Heart rate (beats/min)	LVSP (mm Hg)	LVEDP (mm Hg)	dP/dt_{max} (mm Hg/s)
Sham (n = 7)	123 ± 5	439 ± 12	161 ± 7	2.8 ± 0.6	11,066 ± 312
MI + vehicle (n = 10)	96 ± 3†	436 ± 7	121 ± 3†	19.4 ± 1.5†	7,379 ± 282†
MI + tezosentan (n = 11)	93 ± 2†	432 ± 12	114 ± 3†	8.8 ± 0.7†§	7,700 ± 243†

†$p < 0.001$ vs. Sham; §$p < 0.001$ vs. MI + vehicle.
dP/dt_{max} = maximal rate of rise of left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial pressure; MI = myocardial infarction.

slices of the LV were measured. The entire length of the epicardial circumference and the segment of the epicardial circumference represented by the infarcted segment of each of the four slices of the LV were measured. These were then averaged for each of the four slices. The fraction of the infarcted ventricle was calculated as the average of the four slices expressed as a percent of the length of circumference.

Statistical analysis. All data are presented as mean ± SEM. Statistical analysis was performed by analysis of variance using Statistica (StatSoft) for assessing the differences in variables between groups. Significant differences were then subjected to post-hoc analysis using the Student-Newman-Keuls procedure. Statistical significance was defined as $p < 0.05$. Survival analysis was performed by a log-rank test using SAS for assessing the difference between the two groups. Statistical significance was defined by a p value <0.05.

RESULTS

Cardiac hemodynamics at 48 h. Compared with sham-operated rats, vehicle-treated MI rats developed HF, which was characterized by a highly significant increase in LVEDP with mean values of 19.4 ± 1.5 mm Hg versus 2.8 ± 0.6 mm Hg ($p < 0.001$) and by significant decreases in the maximal rate of rise of LV pressure (LV dP/dt_{max}) and MAP (Table 1). Tezosentan administered at 1 h and 24 h after MI significantly reduced LVEDP by 55% compared with vehicle-treated MI rats ($p < 0.001$). Mean arterial pressure tended to be lower in tezosentan-treated compared with vehicle-treated rats. There was no significant effect on LV dP/dt_{max} (Table 1).

Lung weight and morphology. Baseline values of BW were similar in all groups before surgery. At 48 h after MI, vehicle-treated rats had a significantly lower BW compared with sham-operated rats. Tezosentan significantly reduced the BW loss compared with vehicle-treated rats. Lung weight and lung weight/BW increased compared with sham-operated animals, suggesting that pulmonary edema occurred in the acute phase following coronary artery ligation. Treatment with tezosentan at 1 h and 24 h attenuated the increase in lung weight and lung weight/BW at 48 h compared with vehicle-treated rats (Fig. 1 and Fig. 2).

In this set of experiments, tezosentan had no effect on infarct size (38 ± 2% vs. 41 ± 1% in MI + Veh) at 48 h after MI.

Long-term survival study. At baseline, there was no significant difference in BW among the three groups of animals.

BODY AND HEART WEIGHT. Coronary artery ligation induced a significant decrease in BW (293 ± 10 g vs. 399 ± 15 g; $p < 0.001$) and marked increases in HW/BW, RV/BW and LV+S/BW compared with sham-operated animals. Tezosentan significantly prevented decrease in BW (335 ± 9 g vs. 293 ± 10 g; $p < 0.01$) and the increase in heart weight and RV weight (Table 2). Heart weight and LV and RV weight were significantly reduced by tezosentan when corrected by BW.

INFARCT SIZE. There was no significant difference in infarct size between tezosentan-treated rats and vehicle-treated rats (36 ± 1% vs. 38 ± 1%).

SURVIVAL. The mortality during surgery or within 1 h post-ligation was <1%. During the five-month observation period, none of the sham-operated rats died. The five-

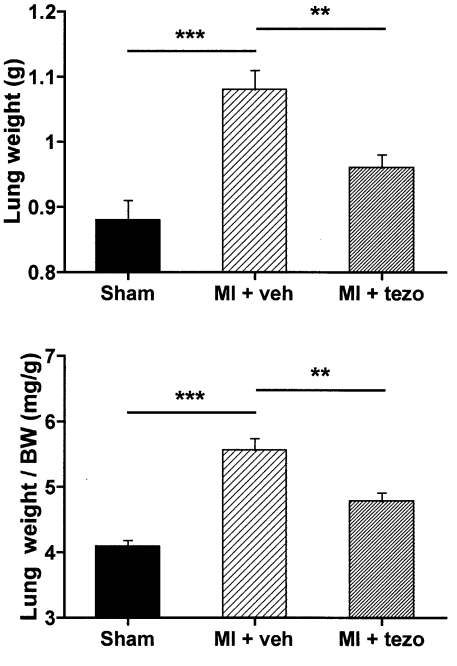

Figure 1. Effects of tezosentan on lung weights at 48 h after myocardial infarction (MI) or sham operation. Tezosentan (10 mg/kg, IV; MI + tezo) or vehicle (saline, 1 ml/kg, IV; MI + veh) was injected at 1 h and 24 h after MI. Sham-operated rats (Sham) received vehicle at the same time points. Lung weight was measured at 48 h after MI or sham operation. BW = body weight; **$p < 0.01$; ***$p < 0.001$.

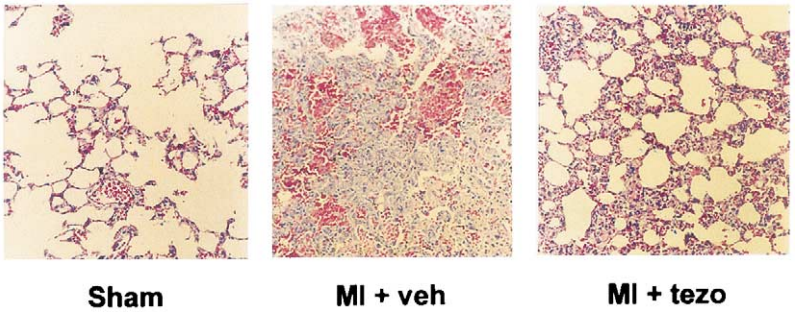

Figure 2. Light micrographs of the lungs at 48 h after myocardial infarction (MI) or sham operation. Tezosentan (10 mg/kg, IV; MI + tezo) or vehicle (saline, 1 ml/kg, IV; MI + veh) was injected at 1 h and 24 h after MI. Tezosentan largely prevented the lung damage (overt capillary dilation and eosin-like edematous materials) seen in the vehicle-treated MI rats (MI + veh). In sham-operated rats (Sham), no pulmonary capillary dilation or edema was observed at 48 h after operation. Hematoxillin eosin stain, × 100.

month survival of vehicle-treated rats with MI was 19%. Administration of tezosentan at 1 h and 24 h after coronary artery ligation significantly increased the long-term survival. The five-month survival of rats treated with tezosentan was 50%, compared with 19% in vehicle-treated rats ($p < 0.001$) (Fig. 3).

DISCUSSION

This study demonstrates that acute and early administration of the dual ET receptor antagonist tezosentan prevents the development of HF and pulmonary edema and significantly increases the survival rate after MI in rats. The rat model of MI by coronary artery ligation is well characterized. The pattern of evolution to overt HF in rats shares many of the features of CHF in humans. Hemodynamic measurements at 48 h after coronary artery ligation show depressed cardiac performance. The increase in lung weight and pulmonary edema may be due to diastolic dysfunction that was favorably modified by tezosentan. Indeed, tezosentan decreased LVEDP without having any significant effect on HR and on cardiac contractility (dP/dt_{max}). Furthermore, tezosentan largely prevented significant increase in lung weight and morphological signs of pulmonary edema in the acute phase of HF. Finally, tezosentan reduced the development of cardiac hypertrophy.

Long-term effects of tezosentan. The main finding of this study is the prevention of long-term mortality after MI by early and short-term treatment with an ET receptor antagonist. Indeed, tezosentan decreased by 51% the five-month mortality of rats subjected to coronary artery ligation. Long-term treatment with the dual ET receptor antagonist bosentan has been shown to improve survival after MI (9). The effect of bosentan was associated with a decrease in LV dilation, cardiac hypertrophy and fibrosis, and with a decrease in BP and a moderate decrease in HR. In this study, treatment with bosentan was initiated seven days after MI. In other studies, bosentan initiated early after MI was also associated with a reduction in LV dilation (14,15). The present study confirms the beneficial effect of a dual ET receptor antagonist given early after MI. Moreover, it is the first study showing the long-term potential of an acute and short-term treatment with a dual ET receptor antagonist after MI.

The mechanism of this increased survival is most likely related to the prevention of the effects of ET-1. Endothelin-1 is a potent hypertrophic agent for cardiomyocytes and increases collagen production by cardiac fibroblasts (17). The orally active dual ET receptor antagonist bosentan decreased cardiac hypertrophy in animal models of hypertension (18,19) and HF (9). Bosentan also reduced cardiac hypertrophy induced by chronic norepinephrine infusion (20) and angiotensin II administration (21). In the present study, tezosentan may have prevented the consequences of the early surge in ET-1 that has been shown to occur in the present animal model (5,22) and in humans after MI (8). Because ET-1 plasma levels at three days after MI are considered the most predictive factor of one-year mortality in humans (8), the effect of this initial surge of ET-1 may have long-term consequences. Even though tezosentan treatment was short-lasting, one can speculate that it prevented the initial events leading to HF, such as release of other mediators, coronary and peripheral vasocon-

Table 2. Effects of Tezosentan on Heart Weights After MI or Sham Operation

	BW (g)	HW (g)	RV (g)	LV + S (g)	HW/BW (mg/g)	RV/BW (mg/g)	LV + S/BW (mg/g)
Sham (n = 20)	399 ± 15	1.08 ± 0.04	0.18 ± 0.01	0.75 ± 0.03	2.72 ± 0.04	0.45 ± 0.02	1.87 ± 0.03
MI + vehicle (n = 51)	293 ± 10†	1.70 ± 0.05†	0.34 ± 0.02†	0.85 ± 0.02*	6.15 ± 0.16†	1.25 ± 0.07†	3.01 ± 0.10†
MI + tezosentan (n = 50)	335 ± 9†‡	1.49 ± 0.05†‡	0.27 ± 0.01†§	0.84 ± 0.02*	4.77 ± 0.27†§	0.89 ± 0.04†§	2.61 ± 0.09†‡

*$p < 0.01$, †$p < 0.001$ vs. Sham; ‡$p < 0.01$, §$p < 0.001$ MI + vehicle.
BW = body weight; HW = heart weight; RV = right ventricular weight; LV + S = left ventricular plus septum weight.

JACC Vol. 39, No. 1, 2002
January 2, 2002:142–7

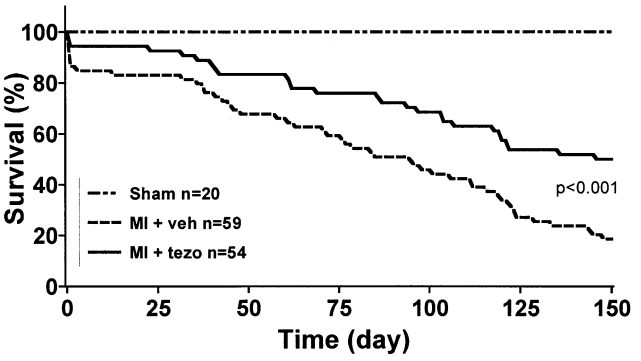

Figure 3. Kaplan-Meier curves showing the effects of tezosentan on survival rate of rats after myocardial infarction (MI) or sham operation. Tezosentan (10 mg/kg, IV; MI + tezo) or vehicle (saline, 1 ml/kg, IV; MI + veh) was injected at 1 h and 24 h after MI. Sham-operated rats (Sham) received vehicle at the same time points.

striction and LV dysfunction. Accordingly, ET receptor blockade was shown to prevent the transition from hypertension to HF (23). Similarly in humans, even though the increase in ET-1 after MI is of short duration, this ET-1 surge seems to have long-term consequences (13).

Another hypothesis is that tezosentan reduced preload acutely, resulting in decreased infarct expansion, progressive wall thickening and chamber dilation.

Effects of tezosentan on pulmonary edema and cardiac function. In addition to its long-term effects of reducing cardiac hypertrophy and mortality at five months after MI, tezosentan exhibited early beneficial effects. The prevention of pulmonary edema after MI by an ET receptor antagonist has not yet been described. Endothelin may indeed contribute to the pathogenesis of pulmonary edema in the acute phase of HF. Elevated plasma ET-1 levels have been reported in pulmonary edema (8,24). Endothelin-1 has pro-inflammatory effects and in particular enhances microvascular permeability (25). It has been shown that ET promotes lung edema and that an ET receptor antagonist reduces the lung edema induced by oleic acid or bicuculline (26–28). Bosentan has been shown to decrease albumin extravasation and to prevent reduction in plasma volume in different models of abnormal vascular permeability (29–31).

Tezosentan treatment also significantly prevented hemodynamic alterations after MI. Tezosentan decreased LVEDP without affecting HR and cardiac contractility (dP/dt_{max}). These results are in agreement with earlier reports showing that ET receptor blockade has a beneficial effect on hemodynamic parameters in CHF (3–5,9–12). The beneficial effects of tezosentan on hemodynamics and pulmonary edema may contribute to the reduced early mortality and the increased long-term survival seen in this study.

Effect of tezosentan on infarct size. In contrast, the beneficial effects of tezosentan on survival are not secondary to a decrease in infarct size. Indeed, tezosentan had no significant effect on infarct size. Generally, in models of MI, ET receptor antagonists act primarily by eliciting coronary vasodilation when a post-ischemic vasoconstriction is observed during reperfusion (32). In MI models, bosentan was shown to be cardioprotective in some studies (33–35) but not others (36,37). Interestingly, in the present study performed in a permanent ischemia model using coronary artery ligation, there was no possibility for reperfusion.

Conclusions. The results of the present study are in agreement with the results of studies performed with early administration of the dual ET_A/ET_B receptor antagonist bosentan. Indeed, long-term bosentan treatment initiated at 3 h (14) or 24 h (15) after MI in rats was associated with a reduction in LV dilation, ventricular dysfunction and ET-1 expression (14,15). The results obtained with the dual ET_A/ET_B receptor antagonists bosentan and tezosentan are in contrast with those obtained after administration of ET_A selective antagonists early after MI. Indeed, early administration of ET_A selective antagonists led to aggravation of LV function and worsening of pulmonary vascular remodeling (38–40). Similarly, delayed (seven days) administration of bosentan led to a marked reduction in mortality at nine months after MI in rats (9), whereas delayed administration of an ET_A selective antagonist did not lead to reduction in mortality in two independent reports (39,41). In a dog model of MI, bosentan-treated, but not BQ-123-treated, dogs exhibited increased cardiac output, suggesting that blockade of ET_B receptors alone or blockade of both ET_A and ET_B receptors is necessary to improve cardiac function in dogs also (42). Overall, there seems to be a discrepancy of effects between dual blockade of ET_A and ET_B receptors and selective blockade of ET_A receptors, especially when treatment is initiated early after MI.

In conclusion, our results demonstrate that acute and short-term treatment with the dual ET receptor antagonist tezosentan markedly increased the long-term survival in a rat model of MI. This observation suggests that tezosentan could be a useful therapeutic agent in the prevention of long-term consequences of MI.

Acknowledgments

The authors gratefully acknowledge the excellent technical assistance of Shuang-Shuang Ding and Jiang-Fei Xi, and thank Alexandra Zürrer for secretarial help in preparing this manuscript.

Reprint requests and correspondence: Dr. Martine Clozel, Actelion Pharmaceuticals Ltd., Innovation Center, Gewerbestrasse 16, CH-4123 Allschwil, Switzerland. E-mail: martine.clozel@actelion.com.

REFERENCES

1. Love MP, McMurray JJ. Endothelin in chronic heart failure: current position and future prospects. Cardiovasc Res 1996;31:665–74.
2. Roux S, Breu V, Ertel SI, Clozel M. Endothelin antagonism with bosentan: a review of potential applications. J Mol Med 1999;77:364–76.

3. Shimoyama H, Sabbah HN, Borzak S, et al. Short-term hemodynamic effects of endothelin receptor blockade in dogs with chronic heart failure. Circulation 1996;94:779–84.
4. Spinale FG, Walker JD, Mukherjee R, Iannini JP, Keever AT, Gallagher KP. Concomitant endothelin receptor subtype-A blockade during the progression of pacing-induced congestive heart failure in rabbits: Beneficial effects on left ventricular and myocyte function. Circulation 1997;95:1918–29.
5. Teerlink JR, Loffler BM, Hess P, Maire JP, Clozel M, Clozel JP. Role of endothelin in the maintenance of blood pressure in conscious rats with chronic heart failure: Acute effects of the endothelin receptor antagonist Ro 47-0203 (bosentan). Circulation 1994;90:2510–8.
6. Tsutamoto T, Wada A, Maeda Y, Adachi T, Kinoshita M. Relation between endothelin-1 spillover in the lungs and pulmonary vascular resistance in patients with chronic heart failure. J Am Coll Cardiol 1994;23:1427–33.
7. Pacher R, Stanek B, Hulsmann M, et al. Prognostic impact of big endothelin-1 plasma concentrations compared with invasive hemodynamic evaluation in severe heart failure. J Am Coll Cardiol 1996;27: 633–41.
8. Omland T, Lie RT, Aakvaag A, Aarsland T, Dickstein K. Plasma endothelin determination as a prognostic indicator of 1-year mortality after acute myocardial infarction. Circulation 1994;89:1573–9.
9. Mulder P, Richard V, Derumeaux G, et al. Role of endogenous endothelin in chronic heart failure: effect of long-term treatment with an endothelin antagonist on survival, hemodynamics, and cardiac remodeling. Circulation 1997;96:1976–82.
10. Sakai S, Miyauchi T, Kobayashi M, Yamaguchi I, Goto K, Sugishita Y. Inhibition of myocardial endothelin pathway improves long-term survival in heart failure. Nature 1996;384:353–5.
11. Kiowski W, Sutsch G, Hunziker P, et al. Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. Lancet 1995;346:732–6.
12. Sutsch G, Kiowski W, Yan XW, et al. Short-term oral endothelin-receptor antagonist therapy in conventionally treated patients with symptomatic severe chronic heart failure. Circulation 1998;98:2262–8.
13. Qiu S, Theroux P, Marcil M, Solymoss BC. Plasma endothelin-1 levels in stable and unstable angina. Cardiology 1993;82:12–9.
14. Fraccarollo D, Hu K, Galuppo P, Gaudron P, Ertl G. Chronic endothelin receptor blockade attenuates progressive ventricular dilation and improves cardiac function in rats with myocardial infarction: possible involvement of myocardial endothelin system in ventricular remodeling. Circulation 1997;96:3963–73.
15. Oie E, Bjonerheim R, Grogaard HK, Kongshaug H, Smiseth OA, Attramadal H. ET-receptor antagonism, myocardial gene expression, and ventricular remodeling during CHF in rats. Am J Physiol 1998;275:H868–77.
16. Clozel M, Ramuz H, Clozel JP, et al. Pharmacology of tezosentan, new endothelin receptor antagonist designed for parenteral use. J Pharmacol Exp Ther 1999;290:840–6.
17. Guarda E, Katwa LC, Myers PR, Tyagi SC, Weber KT. Effects of endothelins on collagen turnover in cardiac fibroblasts. Cardiovasc Res 1993;27:2130–4.
18. Karam H, Heudes D, Bruneval P, et al. Endothelin antagonism in end-organ damage of spontaneously hypertensive rats: Comparison with angiotensin-converting enzyme inhibition and calcium antagonism. Hypertension 1996;28:379–85.
19. Karam H, Heudes D, Hess P, et al. Respective role of humoral factors and blood pressure in cardiac remodeling of DOCA hypertensive rats. Cardiovasc Res 1996;31:287–95.
20. Kaddoura S, Firth JD, Boheler KR, Sugden PH, Poole-Wilson PA. Endothelin-1 is involved in norepinephrine-induced ventricular hypertrophy in vivo: Acute effects of bosentan, an orally active, mixed endothelin ETA and ETB receptor antagonist. Circulation 1996;93: 2068–79.
21. Herizi A, Belabbas H, Mimran A, Jover B. [Cardiac and vascular hypertrophy in hypertension due to angiotensin II: Effect of losartan and bosentan]. Arch Mal Coeur Vaiss 2000;93:983–6.
22. Sakai S, Miyauchi T, Sakurai T, et al. Endogenous endothelin-1 participates in the maintenance of cardiac function in rats with congestive heart failure: Marked increase in endothelin-1 production in the failing heart. Circulation 1996;93:1214–22.
23. Iwanaga Y, Kihara Y, Hasegawa K, et al. Cardiac endothelin-1 plays a critical role in the functional deterioration of left ventricles during the transition from compensatory hypertrophy to congestive heart failure in salt-sensitive hypertensive rats. Circulation 1998;98:2065–73.
24. Langleben D, DeMarchie M, Laporta D, Spanier AH, Schlesinger RD, Stewart DJ. Endothelin-1 in acute lung injury and the adult respiratory distress syndrome. Am Rev Respir Dis 1993;148:1646–50.
25. Filep JG, Sirois MG, Rousseau A, Fournier A, Sirois P. Effects of endothelin-1 on vascular permeability in the conscious rat: interactions with platelet-activating factor. Br J Pharmacol 1991;104:797–804.
26. Ishizaki T, Shigemori K, Ameshima S, et al. Protective effects of BQ-123, an ETA receptor antagonist, against leukotoxin-induced injury in rat lungs. Am J Physiol 1996;271:459–63.
27. Ishizaki T, Shigemori K, Nakai T, et al. Endothelin-1 potentiates leukotoxin-induced edematous lung injury. J Appl Physiol 1995;79: 1106–11.
28. Herbst C, Tippler B, Shams H, Simmet T. A role for endothelin in bicuculline-induced neurogenic pulmonary oedema in rats. Br J Pharmacol 1995;115:753–60.
29. Filep JG, Clozel M, Fournier A, Foldes-Filep E. Characterization of receptors mediating vascular responses to endothelin-1 in the conscious rat. Br J Pharmacol 1994;113:845–52.
30. Filep JG. Endogenous endothelin modulates blood pressure, plasma volume, and albumin escape after systemic nitric oxide blockade. Hypertension 1997;30:22–8.
31. Filep JG. Role for endogenous endothelin in the regulation of plasma volume and albumin escape during endotoxin shock in conscious rats. Br J Pharmacol 2000;129:975–83.
32. Seo B, Oemar BS, Siebenmann R, von Segesser L, Luscher TF. Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels. Circulation 1994;89:1203–8.
33. Li XS, Wang QD, Pernow J. Beneficial effects of the endothelin receptor antagonist bosentan on myocardial and endothelial injury following ischaemia/reperfusion in the rat. Eur J Pharmacol 1995;283: 161–8.
34. Wang QD, Li XS, Lundberg JM, Pernow J. Protective effects of non-peptide endothelin receptor antagonist bosentan on myocardial ischaemic and reperfusion injury in the pig. Cardiovasc Res 1995;29: 805–12.
35. Wang QD, Li XS, Pernow J. The nonpeptide endothelin receptor antagonist bosentan enhances myocardial recovery and endothelial function during reperfusion of the ischemic rat heart. J Cardiovasc Pharmacol 1995;26:S445–7.
36. Dagassan PH, Breu V, Clozel M, Clozel JP. Role of endothelin during reperfusion after ischemia in isolated perfused rat heart. J Cardiovasc Pharmacol 1994;24:867–74.
37. Richard V, Kaeffer N, Hogie M, Tron C, Blanc T, Thuillez C. Role of endogenous endothelin in myocardial and coronary endothelial injury after ischaemia and reperfusion in rats: studies with bosentan, a mixed ETA-ETB antagonist. Br J Pharmacol 1994;113:869–76.
38. Hu K, Gaudron P, Schmidt TJ, Hoffmann KD, Ertl G. Aggravation of left ventricular remodeling by a novel specific endothelin ET(A) antagonist EMD94246 in rats with experimental myocardial infarction. J Cardiovasc Pharmacol 1998;32:505–8.
39. Nguyen QT, Cernacek P, Calderoni A, et al. Endothelin A receptor blockade causes adverse left ventricular remodeling but improves pulmonary artery pressure after infarction in the rat. Circulation 1998;98:2323–30.
40. Nguyen QT, Colombo F, Rouleau JL, Dupuis J, Calderone A. LU135252, an endothelin(A) receptor antagonist, did not prevent pulmonary vascular remodelling or lung fibrosis in a rat model of myocardial infarction. Br J Pharmacol 2000;130:1525–30.
41. Mulder P, Richard V, Thuillez C. Endothelin antagonism in experimental ischemic heart failure: hemodynamic, structural and neurohumoral effects. Heart Fail Rev 2001;6:295–300.
42. Ohta H, Suzuki J, Akima T, Kawai N, Hanada K, Nishikibe M. Hemodynamic effect of endothelin antagonists in dogs with myocardial infarction. J Cardiovasc Pharmacol 1998;31:S255–7.