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Effects of a new endothelin receptor antagonist, TAK-044, on myocardial stunning in dogs

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Abstract

The effects of a new endothelin receptor 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 salt, on ischemic and post-ischemic myocardial dysfunction (stunned myocardium) were studied in anesthetized open-chest dogs. A short (15 min) occlusion of the left anterior descending coronary artery followed by 5-h reperfusion significantly reduced myocardial segment shortening during and after the ischemic period in the ischemic region. Regional myocardial blood flow was also decreased significantly 10 min after the occlusion, whereas it returned almost completely to its pre-ischemic value 5 h after reperfusion. TAK-044 (3 mg/kg,i.v.) administered 10 min before occlusion significantly improved the reduced myocardial segment shortening in the ischemic region during and after occlusion. Cardiovascular hemodynamics and regional myocardial blood flow in a TAK-044-treated group were identical to those in the control group. These results indicate that endogenous endothelin contributes to the cause of ischemic and post-ischemic myocardial dysfunction without changing either hemodynamics or regional myocardial blood flow.

Keywords: Endothelin; Myocardium, stunned; TAK-044; (Dog)

1. Introduction

Stunning is defined as a fully reversible dysfunction of the reperfused myocardium after brief periods of ischemia without histological and metabolic signs of irreversible injury (Braunwald and Kloner, 1982). The paradox of this long-lasting dysfunction in completely viable tissue has attracted attention, so that many experimental studies have been reported that mostly used a canine brief ischemia-reperfusion model. Stunning is now considered to be an underlying phenomenon in some types of dysfunction in coronary artery disease and related conditions in humans (Bolli et al., 1989a,b; Heyndrickx and Paulus, 1990).

Endothelin-1 has been reported to contribute to ischemic heart disease (Miyauchi et al., 1989; Matsuyama et al., 1991; Toyo-oka et al., 1991; Spielberg et al., 1991). In addition to its coronary vasoconstrictive effects, endothelin-1 showed a direct action on cardiac contractility (Watanabe et al., 1989; Wang et al., 1991b; Wang and Morgan, 1992) and extends myocardial infarction. We have shown that a monoclonal antibody against endothelin-1 and endothelin receptor antagonist reduced infarct size in a rat ischemia-reperfusion model in which plasma and cardiac endothelin-1 increased with time after ischemia (Watanabe et al., 1991). Regarding myocardial stunning induced by short ischemia, Hayashida et al. (1992) reported that endothelin-1 infusion impaired the recovery of myocardial shortening during reperfusion after 10-min coronary occlusion in conscious dogs, which suggests a detrimental effect of endothelin-1 on myocardial stunning. However, there is no direct evidence for the pathophysiological involvement of endothelin-1 in the stunned myocardium.

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 salt is an ET_A/ET_B endothelin receptor antagonist and has potent affinity for both ET_A and ET_B receptors (Ikeda et al., 1994). It antagonizes contractile responses to endothelin-1 in coronary, renal,

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mesentery and femoral arteries in vitro (Awane et al., 1994), and suppresses the hemodynamic effects of endothelin-1 in vivo (Watanabe et al., 1995). In addition, it reduces infarct size in an ischemia-reperfusion myocardial infarction model in rats(Watanabe et al., 1995). Thus we designed the present study to assess the pathophysiological role of endogenous endothelin-1 in myocardial stunning, using an endothelin antagonist, TAK-044, in anesthetized dogs.

2. Materials and methods

2.1. General preparation

Adult male beagle dogs (11–15 kg) were anesthetized with sodium pentobarbital (35 mg/kg, i.v.) and ventilated(Model 701, Harvard Apparatus, South Natick, MA, USA). Body temperature was maintained at 38°C with a heating pad. Throughout the experiment, blood pH, pO_2 and pCO_2 were maintained at physiological levels by adjusting the respiratory rate and volume. Aortic blood pressure and left ventricular pressure were monitored by inserting a polyethylene catheter into the aorta via the femoral artery and a pressure transducer-tipped catheter (MPC-500, Millar Instruments, Houston, TX, USA) into the left ventricle via the carotid artery, respectively. The dP/dt of left ventricular pressure was measured electronically. The right femoral vein was cannulated for the administration of drugs. A left thoracotomy was performed at the fifth intercostal space, and the heart was exposed. A portion of the left anterior descending coronary artery was isolated distal to the first diagonal branch and an electromagnetic flow probe (Skalar, Utolehito, Netherlands) was placed around the vessel for the measurement of left anterior descending coronary artery blood flow. A 5-0 silk thread, an occluder, was placed distal to the flow probe. All measurements including segment shortening were monitored on a polygraph (model RM-6000, Nihon Khoden, Tokyo, Japan) and analyzed with a MacLab system (MacLab/8, AD Instruments, Castle Hill, Australia).

Fifteen-min occlusion of the left anterior descending coronary artery followed by 5-h reperfusion was performed. Three mg/kg of TAK-044 or saline (vehicle) was given intravenously 10 min before occlusion. Blood samples (2.5 ml) for the measurement of plasma endothelin-1 were obtained from the femoral vein 10 min before and 14 min after the occlusion, and 5 and 30 min after reperfusion in the control group. Blood samples for the measurement of plasma creatine phosphokinase MB isoenzyme release were collected 14 min after left anterior descending coronary artery occlusion, and 30 min and 5 h after reperfusion. The plasma concentration of endothelin-1 was measured with the ELISA method (Suzuki et al., 1989). The creatine phosphokinase MB isoenzyme was separated by electrophoresis, and measured using a kit (Nescoat CK, Nihon Syoji, Osaka, Japan).

2.2. Myocardial segment shortening

Regional myocardial segment function (percentage segment shortening, %SS) was measured in the regions perfused by the left anterior descending coronary artery and by the left circumflex coronary artery using two sets of piezoelectric crystals (5 MHz). Precalibrated crystals were inserted into the middle position between epicardium and endocardium (approximately 5 mm apart and 5 mm deep). The leads of each crystal were connected to ultrasonic amplifiers (Triton, Houston, TX, USA). Changes in transmission time, which indicate the distance between the two crystals, were monitored using an oscilloscope (model 520, Panasonic, Osaka, Japan). Systolic segment length (SL) was measured at peak negative LV$dP/dt$ and diastolic segment length (DL) was measured at the onset of the rapid rise of positive LV$dP/dt$. %SS was calculated with the equation: $\%SS = (DL - SL) \times 100/DL$. On completion of each experiment, the depth of the crystals was verified histologically.

2.3. Myocardial tissue blood flow

Regional myocardial blood flow was determined by means of the microsphere technique. The left atrial appendage and the left femoral artery were cannulated for the administration of microspheres and for the withdrawal of a reference blood flow sample, respectively. Approximately 5×10^6 colored microspheres (15 ± 0.3 μm diameter, Primtec, EZ-TRAC, Tokyo, Japan) were injected into the left atrium followed by 1 ml saline wash. Five seconds before sphere administration, a reference blood flow sample was obtained from the femoral artery at a constant rate, 8.6 ml/min, for 70 s. On completion of each experiment, the left anterior descending coronary artery was ligated at the site of previous occlusion, and Evans blue was injected into the coronary vasculature through the left atrial catheter to delineate the ischemic region. Subsequently, the heart was removed and sectioned into transmural blocks of both the non-ischemic (left circumflex coronary artery) and ischemic (left anterior descending coronary artery) regions. The tissue blocks (1 g) were weighed and stored in a refrigerator. The following day, the spheres in each block and reference blood flow samples were counted using a counting kit (EZ-TRAC counting kit, Primtec, Tokyo, Japan). Myocardial blood flow was calculated from the following formula as transmural myocardial blood flow:

$$Q_m = (C_m \times Q_r)/C_r$$

Q_m: regional myocardial blood flow (ml/min/g tissue)
C_m: numbers of microspheres/g tissue
Q_r: sampling rate of reference blood flow sample (8.6 ml/min)

Table 1
Baseline values of cardiovascular hemodynamics

	Control ($n=7$)	TAK-044 ($n=6$)
MBP (mm Hg)	131 ± 7	125 ± 4
CBF (ml/min)	10.1 ± 0.9	8.5 ± 1.1
HR (bpm)	167 ± 6	164 ± 10
LVSP (mm Hg)	143 ± 6	131 ± 4
LV + dP/dt (mm Hg/s)	3934 ± 427	3472 ± 384

MBP, mean arterial blood pressure; CBF, blood flow of left anterior descending coronary artery; HR, heart rate; LVSP, left ventricular systolic pressure; LV + dP/dt, maximum values of positive dP/dt of left ventricular pressure. Data are means ± S.E.

C_r: numbers of microspheres in the reference blood flow sample

2.4. Drugs and statistical analyses

TAK-044 was dissolved in saline at a concentration of 30 mg/ml. All data are presented as means ± S.E. To clarify the changes in value of each parameter before and after ischemia, all data were converted into percentages of pre-ischemic baseline values (shown in Tables 1 and 2). The effects of TAK-044 during both ischemia and reperfusion periods were evaluated statistically with a repeated measures analysis of variance (ANOVA) with Greenhous-Geisser correction using a computer program (SAS, SAS Institute, NC, USA). A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Myocardial segment shortening

In the control group, occlusion of the left anterior descending coronary artery caused significant decreases in regional myocardial segment shortening of the ischemic region (Fig. 1a). In the non-ischemic region, there was no significant change in %SS duringthe experimental period (Fig. 1b). Decreases in the %SS during the ischemic period

Table 2
Baseline values of myocardial segment length and percentage segment shortening

	Control ($n=7$)	TAK-044 ($n=6$)
DL (IS) (mm)	5.85 ± 0.81	4.81 ± 0.41
SL (IS) (mm)	4.54 ± 0.56	3.87 ± 0.35
DL (NS) (mm)	5.05 ± 0.49	5.24 ± 0.42
SL (NS) (mm)	4.48 ± 0.42	4.77 ± 0.40
%SS (IS) (%)	19.2 ± 3.4	15.9 ± 3.6
%SS (NS) (%)	9.0 ± 1.8	7.2 ± 1.5

DL, diastolic segment length; SL, systolic segment length; %SS, percentage segment shortening calculated by (DL − SL) × 100/DL; IS, ischemic region; NS, non-ischemic region. Data are means ± S.E.

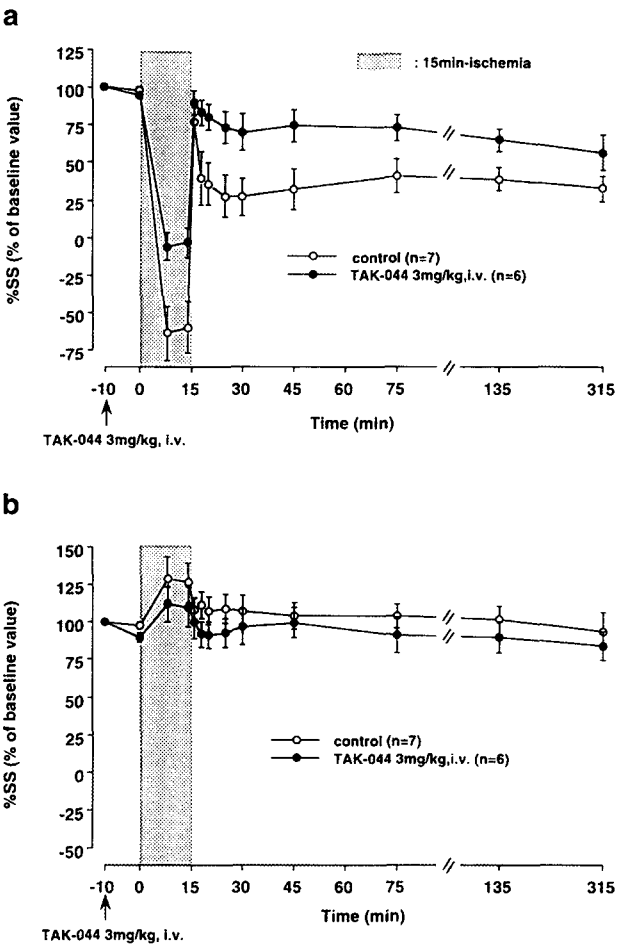

Fig. 1. Time courses of changes in segment shortening (%SS) with ischemia and reperfusion of the left anterior descending coronary artery. Fig. 1a shows the changes in %SS of the ischemic (left anterior descending coronary artery) region and Fig. 1b shows the changes in %SS of the non-ischemic (left circumflex coronary artery) region. %SS is expressed as percent of pre-ischemic baseline values along the horizontal axis of time after the onset of ischemia. The hatched area indicates 15 min of ischemia. TAK-044 was administered intravenously 10 min before ischemia, as indicated by an arrow. Data were expressed as means ± S.E. During and after ischemia, significant differences ($P < 0.05$) between TAK-044-treated and control group were detected in a repeated measures ANOVA.

were attenuated significantly by TAK-044 compared to those in the control group (−60.3 ± 17.2%, $n=7$, and −4.0 ± 9.9%, $n=6$, at 14 min after occlusion in the control and TAK-044 treated groups, respectively). After reperfusion, a significant attenuation of the decreases in %SS by TAK-044 was also observed (27.3 ± 14.4%, $n=7$, and 73.0 ± 11.0%, $n=6$, at 10 min after reperfusion in the control and TAK-044-treated groups, respectively). In the non-ischemic region, there was no significant difference in changes in %SS between the TAK-044-treated group and the control group. TAK-044 (3 mg/kg, i.v.) had no significant effects on the %SS at 10 min after its administration (just before occlusion) in either region (Fig. 1a,b).

3.2. Cardiovascular hemodynamics

In the control group, 15-min occlusion caused a slight but significant decrease in mean blood pressure and left ventricular systolic pressure during the ischemic period, but both returned to their pre-ischemic values within 30 min after reperfusion (Fig. 2a,b). The left ventricular positive dP/dt decreased significantly (78.1 ± 3.7%, $n = 7$, at 14 min after occlusion) and remained at a lower value than the pre-ischemic value throughout the experimental period (Fig. 2c). After reperfusion, a marked reactive hyperemia of left anterior descending coronary artery blood flow was observed (Fig. 2d), but the flow returned to its pre-ischemic value within 30 min after reperfusion. The heart rate was slightly, but significantly increased after reperfusion (Fig. 2e).

TAK-044 (3 mg/kg, i.v.) itself had no significant effect on the cardiovascular hemodynamics 10 min after its administration (justbefore occlusion). The cardiovascular hemodynamics in the TAK-044-treated group were almost identical to those in the control group except for left ventricular systolic pressure and heart rate. In the TAK-044-treated group, a slight but significant ($P < 0.05$) decrease in left ventricular systolic pressure and increase in heart rate compared to those in the control group were observed after reperfusion. The degree of reactive hyperemia of left anterior descending coronary artery blood flow in the TAK-044-treated group was relatively small (620 ± 95%, $n = 7$, and 414 ± 89%, $n = 6$, of pre-ischemic value at 1 min after reperfusion in the control and TAK-044-treated group, respectively), but not significantly different from that in the control group.

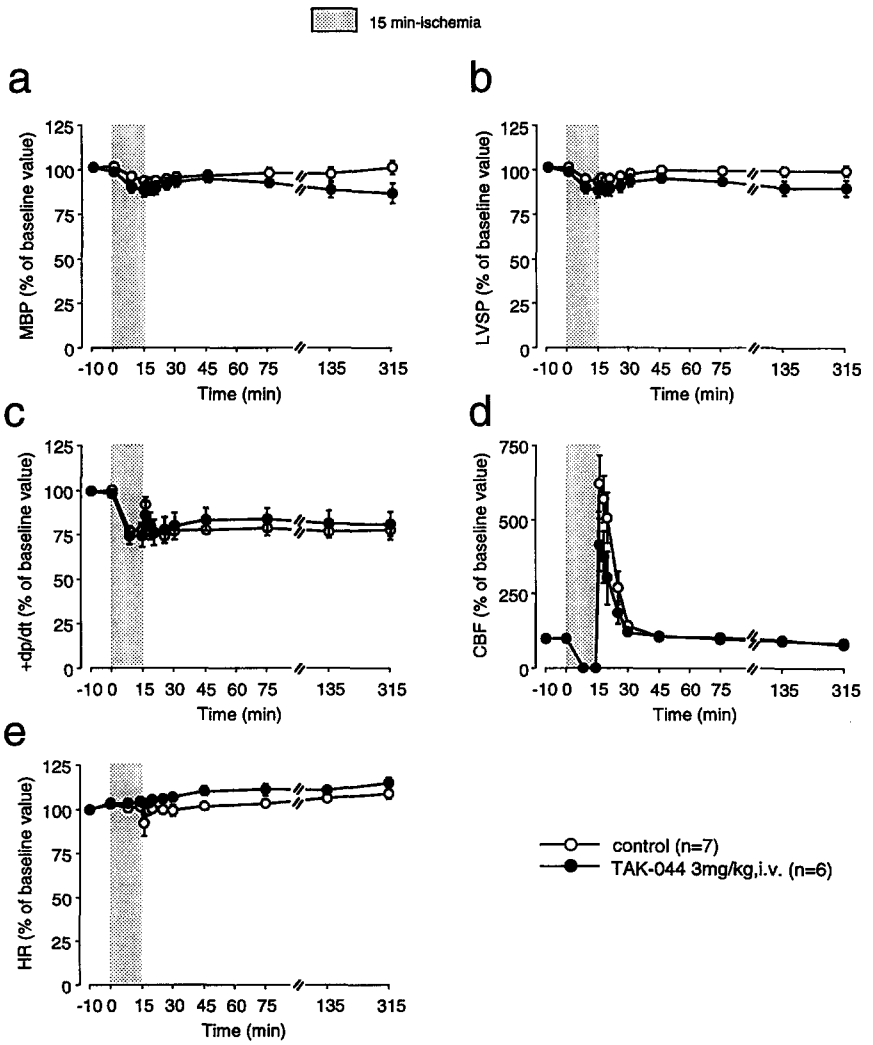

Fig. 2. Time courses of changes in hemodynamics with ischemia and reperfusion of the left anterior descending coronary artery. MBP, mean arterial blood pressure (a); LVSP, left ventricular systolic pressure (b); $+dP/dt$, maximum values for positive dP/dt of left ventricular pressure (c); CBF, blood flow of left anterior descending coronary artery (d); HR, heart rate (e). All data were expressed as percentages of pre-ischemic baseline values along the horizontal axis of time after the onset of ischemia. The hatched area indicates 15 min of ischemia. TAK-044 was administered intravenously 10 min before ischemia, as indicated by an arrow. Data were expressed as means ± S.E. After reperfusion, significant differences ($P < 0.05$) between TAK-044-treated and control group were observed for left ventricular systolic pressure and heart rate with the same statistical analysis as in Fig. 1.

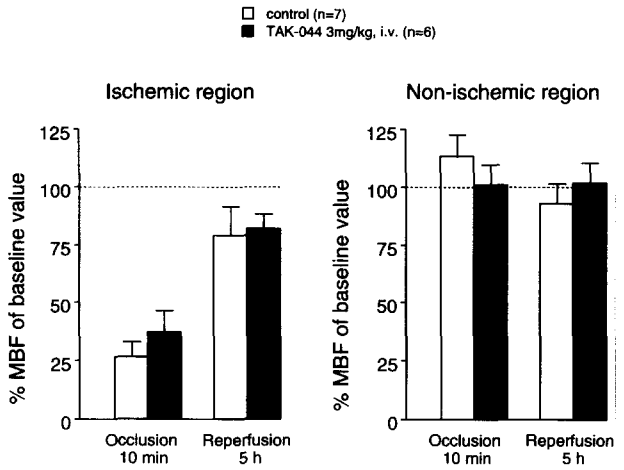

Fig. 3. Changes in regional myocardial blood flow (MBF) in dogs with ischemia and reperfusion of the left anterior descending coronary artery. The data were obtained 15 min before ischemia (baseline), 10 min after the onset of ischemia and 5 h after reperfusion. All data were expressed as percentages of pre-ischemic baseline values. Data were expressed as means ± S.E.

3.3. Plasma endothelin-1

No significant change in plasma endothelin-1 level was observed. The plasma endothelin-1 level in the control group was 1.9 ± 0.3, 2.3 ± 0.4, 2.2 ± 0.5 and 2.3 ± 0.6 pg/ml pre-ischemic, 14 min after occlusion and 5 min and 30 min after reperfusion, respectively ($n = 7$).

3.4. Regional myocardial blood flow

The baseline data for regional myocardial blood flow in the ischemic (left anterior descending coronary artery) region in the control and TAK-044-treated group were 0.86 ± 0.17 ml/min/g tissue, $n = 7$, and 0.73 ± 0.10 ml/min/g tissue, $n = 6$, respectively. These values for the non-ischemic (left circumflex coronary artery) region were 1.11 ± 0.12 ml/min/g, $n = 7$, in the control group and 0.80 ± 0.09 ml/min/g, $n = 6$, in the TAK-044-treated group. The regional myocardial blood flow in the ischemic region was decreased significantly 10 min after occlusion in the control group, whereas it recovered to more than 80% of its pre-ischemic value 5 h after reperfusion (Fig. 3, control). In the non-ischemic region, no significant changes in myocardial blood flow were observed during either ischemic or reperfusion period (Fig. 3 right).

In the TAK-044-treated group, there were regional myocardial blood flow changes in the ischemic region similar to those in the control group. There were no significant differences in the regional myocardial blood flow changes between control and TAK-044-treated groups throughout the experimental period.

3.5. Validity of the stunned myocardial model

Three out of 16 animals (2 out of 9 in the control group and 1 out of 8 in the TAK-044-treated group) had ventricular fibrillation within 10 min after reperfusion and died. The baseline data for hemodynamics, myocardial segment length and percent segment shortening of survivors in two groups are shown in Tables 1 and 2, respectively. There were no significant differences in these parameters between the control group and the TAK-044-treated group. Fifteen min of coronary artery occlusion produced no detectable plasma creatine phosphokinase MB isoenzyme. Since plasma creatine phosphokinase MB isoenzyme level can be used as a marker of irreversible myocardial damage, our finding suggests that there was no irreversible myocardial damage in our experiments, and is consistent with the results of Bolli (1990) and of others (Charlat et al., 1989).

4. Discussion

In the present study, we demonstrated that the endothelin receptor antagonist, TAK-044, attenuated regional myocardial dysfunction during and after a brief period (15 min) of ischemia in anesthetized dogs. This suggested that endogenous endothelin plays a role in this experimental condition. Since myocardial blood flow was restored after a brief period of ischemia and there was no detectable irreversible myocardial damage (necrosis) in our study, the regional myocardial dysfunction after a brief period of ischemia meets the definition of myocardial stunning. Thus, endogenous endothelin may be involved in the pathogenesis of stunned myocardium.

Improvement of regional myocardial function (%SS) in the ischemic and post-ischemic region could be achieved by the following mechanisms. Positive inotropic intervention can improve myocardial stunning (Bolli, 1990). Myocardial energy demand related to hemodynamics and blood flow supply are important factors in ischemia-induced myocardial dysfunction (Dunn and Griggs, 1975; Palacios et al., 1978; Kumada et al., 1979). A hemodynamic change-induced reduction in the cardiac work load decreases myocardial energy consumption and also has an indirect protective effect on the myocardium exposed to ischemia. A decrease in heart rate has similar effects on the ischemic myocardium. An increase in regional myocardial blood flow in the ischemic region is another mechanism responsible for the improvement of myocardial function in the ischemic region.

Since there were no significant changes in regional segment shortening and $+dP/dt$ 10 min after administration of TAK-044 (just before occlusion), TAK-044 itself had no direct effect on these parameters. Thus the improvement of ischemic and post-ischemic regional myocar-

dial dysfunction by TAK-044 was at least not the result of its direct inotropic effects on the myocardium.

During the ischemic period and the early phase of reperfusion, the hemodynamic changes in the controls and the TAK-044-treated dogs were almost identical. Moreover, there was no difference in the calculated double products (left ventricular systolic pressure × heart rate) between the two groups throughout the experiment (data not shown). Thus the protective effects of TAK-044 on ischemic and post-ischemic myocardium were unlikely to the result of a reduction in energy demand because of hemodynamic changes.

In the case of blood flow supply to the myocardium, there was no significant difference in left anterior descending coronary blood flow between the two groups throughout the experimental periods. Regional myocardial blood flow measured at any time points was also identical in the two groups. Thus the improvement of %SS in the ischemic region by TAK-044 was independent of regional myocardial blood flow or coronary blood flow. These lines of evidence indicate that the ameliorating effect of TAK-044 on the regional myocardial function cannot be explained by the improvement of regional myocardial blood flow.

One of the plausible explanations is that pro-ischemic effects of endothelin-1 are responsible for the stunned myocardium. Recently, Hayashida et al. (1992) found that an infusion of endothelin-1, which produced no effect on the function of non-ischemic myocardium, worsened the function of post-ischemic myocardium in conscious dogs. Moreover, Grover et al. (1992) demonstrated that endothelin-1 reduces the time to contracture under ischemic conditions in the isolated rat heart. The results of these studies indicate that endothelin-1 has a pro-ischemic effect, which aggravates the myocardial damage induced by ischemia.

Endothelin-1 not only induces vasoconstriction in coronary vessels but also has direct positive inotropic effects which cause myocardial ischemic damage (Watanabe et al., 1989; Neubauer et al., 1991; Wang et al., 1991b; Wang and Morgan, 1992). Grover et al. (1992) have suggested that the vasoconstrictive action of ET itself can impair myocardial perfusion and induce myocardial ischemia in a similar manner to coronary vasospasm. The present study did not show any differences in regional myocardial blood flow or left anterior descending coronary artery blood flow between control and TAK-044-treated groups, suggesting that vasoconstrictive effects of endothelin-1 play a minor role in the stunned myocardium. Endothelin-1 has been reported to increase cytosolic $[Ca^{2+}]_i$ (Wang et al., 1991a; Wang and Morgan, 1992), and this, in turn, induces Ca^{2+} overload and/or increases energy demand in the myocardium under ischemic conditions (Grover et al., 1992; Schwartz et al., 1991). Since Ca^{2+} overload has been suggested as one of the mechanisms of myocardial stunning (Bolli, 1990), this scheme is attractive to explain the deteriorative role of endothelin-1 on myocardial ischemia.

Several investigators reported that the level of endothelin-1 increases during ischemia in the heart (Miyauchi et al., 1989; Tonnessen et al., 1993; Wang et al., 1995). We also reported that plasma and heart endothelin-1 were increased in the rat (Watanabe et al., 1991) and rabbit (Kusumoto et al., 1993) myocardial infarct model with ischemia-reperfusion. Tonnessen et al. (1993) measured plasma endothelin-1 levels in both coronary arterial and venous blood in pigs with 10-min ischemia-reperfusion and demonstrated an increase in outflow of endothelin-1 from the heart under these conditions. In the present study, we collected the blood sample from the thoracic aorta, because we did not want to catheterize both coronary artery and vein at the risk of damaging the ischemic region. It thus might be difficult to detect a local (heart) increase in endothelin-1 level, had it occurred. Separate studies will be required to clarify the local endogenous production of endothelin-1 in our protocol.

Myocardial stunning has been suggested to be involved in the pathophysiology of acute myocardial infarction and angina (Bolli et al., 1989a,b; Heyndrickx and Paulus, 1990; Clozel and Clozel, 1989; Tonnessen et al., 1993). Prolonged myocardial dysfunction after direct percutaneous transluminal coronary angioplasty is, at least in part, due to myocardial stunning (Bolli et al., 1989a; Miyauchi et al., 1989). Although myocardial stunning is identified as fully reversible myocardial cell damage, the decrease in contractility in a large part of the ventricle produces pump failure and leads to cardiogenic shock. Our results with TAK-044 serve as a new approach in the treatment of this myocardial dysfunction.

In conclusion, endogenous endothelin should be included among the pathogenic causes of stunned myocardium. TAK-044 is expected to be an effective drug in the treatment of ischemic heart disease.

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References

Awane, Y., K. Kusumoto, K. Kubo, A. Kawata, T. Kikuchi, M. Wakimasu, T. Watanabe and M. Fujino, 1994, Pharmacological profile in vivo of a new endothelin antagonist, TAK-044, Jpn. J. Pharmacol. 64, Suppl. I, 167P.

Bolli, R., 1990, Mechanism of myocardial stunning, Circulation 82, 723.

Bolli, R., M.O. Jeroudi, B.S. Patel, O.I. Aruoma, B. Halliwell, E.K. Lai and P.B. MeCay, 1989a, Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial 'stunning' is a manifestation of reperfusion injury, Circ. Res. 65, 807.

Bolli, R., B.S. Patel, C.J. Hartley, J.I. Thornby, M.O. Jeroudi and R. Boberts, 1989b, Nonuniform transmural recovery of contractile function in stunned myocardium, Am. J. Physiol. 257, H375.

Braunwald, E. and R.A. Kloner, 1982, The stunned myocardium: prolonged postischemic ventricular dysfunction, Circulation 66, 1146.

Charlat, M.L., P.G. O'Neill, C.J. Hartley, R. Roberts and R. Bolli, 1989, Prolonged abnormalities of left ventricular diastolic wall thinning in the 'stunned' myocardium in conscious dogs: time-course and relation to systolic function, J. Am. Coll. Cardiol. 13, 185.

Clozel, J.P. and M. Clozel, 1989, Effects of endothelin on the coronary vascular bed in open-chest dogs, Circ. Res. 66, 1193.

Dunn, R.B. and D.M. Griggs, 1975, Transmural gradients in ventricular tissue metabolite produced by stopping coronary flow in the dog, Circ. Res. 37, 438.

Grover, G.J., P.G. Sleph, M. Fox and N.C. Trippodo, 1992, Role of Endothelin-1 and big endothelin-1 in modulating coronary vascular tone, contractile function and severity of ischemia in rat hearts, J. Pharmacol. Exp. Ther. 263, 1074.

Hayashida, W., T. Kumada, F. Kohno, M. Noda, N. Ishikawa, M. Kambayashi and C. Kawai, 1992, Left ventricular relaxation in dilated cardiomyopathy: relation to loading conditions and regional nonuniformity, J. Am. Coll. Cardiol. 20, 1082.

Heyndrickx, G.R. and W.J. Paulus, 1990, Effect of asynchrony on left ventricular relaxation, Circulation 81, Suppl. III, III-41.

Ikeda, S., Y. Awane, K. Kusumoto, M. Wakimasu, T. Watanabe and M. Fujino, 1994, A new endothelin receptor antagonist, TAK-044, shows long-lasting inhibition of both ETA- and ETB-mediated blood pressure responses in rats, J. Pharmacol. Exp. Ther. 270, 728.

Kumada, T., J. S. Karliner, H. Pouleur, K.P. Gallagher, K. Shirato and J. Ross, Jr., 1979, Effects of coronary occlusion on early ventricular diastolic events in conscious dog, Am. J. Physiol. 237, H542.

Kusumoto, K, Y. Awane, S. Fujiwara and T. Watanabe, 1993, Role of endogenous endothelin in extension of rabbit myocardial infarction, J. Cardiovasc. Pharmacol. 22 (Suppl. 8), S339.

Matsuyama, K, H. Yasue, K. Okumura, Y. Saito, K. Nakao, G. Shirakami and H. Imura, 1991, Increased plasma level of endothelin-1-like immunoreactivity during coronary spasm in patients with coronary spastic angina, Am. J. Cardiol. 68, 991.

Miyauchi, T., M. Yanagisawa, T. Tomizawa, Y. Sugishita, N. Suzuki, M. Fujino, R. Ajisaka, K. Goto and T. Masaki, 1989, Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction, Lancet 2, 53.

Neubauer, S., S. Zimmermann, A. Hlrsch, F. Fulzer, N. Tian, W. Bauer, H. Bauer and G. Ertl, 1991, Effects of Endothelin-1 in the isolated heart in ischemia/reperfusion and hypoxia/reoxygenation injury, J. Mol. Cell. Cardiol. 23, 1397.

Palacios, I., J.B. Newell and W.J. Powell Jr., 1978, Effects of acute global ischemia on diastolic relaxation in canine hearts, Am. J. Physiol. 235, H729.

Schwartz, G., G.I.S. Schaefer, S.D. Trochai S. Steinman, J. Gober, J. Garciai B. Massie and M.W. Wiener, 1991, Metabolic and functional consequences of blunted myocardial reactive hyperemia, Am. J. Physiol. 261, H892.

Spielberg, C., K. Schwenn, J. Hensen and T. Linderer, 1991, Endothelin levels in the coronary sinus during PTCA of the left descending coronary artery, Circulation 84, 11726.

Suzuki, N., H. Matsumoto, C. Kitada, T. Masaki and M. Fujino, 1989, A sensitive sandwich-enzyme immunoassay for human endothelin, J. Immunol. Meth. 118, 245.

Tonnessen, T., P.A. Naess, K.A. Kirkebeen, J. Offstad, A. Ilebekki and G. Christensen, 1993, Release of endothelin from the porcine heart after short term coronary artery occlusion, Cardiovasc. Res. 27, 1482.

Toyo-oka, T., T. Aizawa, N. Suzuki, Y. Hirata, T. Miyauchi, W.S. Shin, M. Yanagisawa, T. Masaki and T. Suginoto, 1991, Increased plasma level of endothelin-1 and coronary spasm induction in patients with vasospastic angina pectoris, Circulation 83, 476.

Wang, J.X. and J.P. Morgan, 1992, Endothelin reverses the effects of acidosis on the intracellular Ca^{2+} transient and contractility in ferret myocardium, Circ. Res. 71, 631.

Wang, J.X., G. Paik and J.P. Morgan, 1991a, Endothelin-1 enhances myofilament Ca^{2+} responsiveness in aequorin-loaded ferret myocardium, Circ. Res. 69, 582.

Wang, J., G.A. Zeballos, G. Kaley and T.H. Hintze, 1991b, Dilation and constriction of large coronary arteries in conscious dogs by endothelin, Am. J. Physiol. 261, H1379.

Wang, Q.D., X.S. Li, J.M. Lundberg and J. Pernow, 1995, Protective effects of non-peptide endothelin receptor antagonist bosentan on myocardial ischaemic and reperfusion injury in the pig, Cardiovasc. Res. 29, 805.

Watanabe, T., K. Kusumoto, T. Kitayoshi and N. Shimamoto, 1989, Positive inotropic and vasoconstrictive effects of endothelin-1 in in vivo and in vitro experiments: characteristics and the role of L-type calcium channels, J. Cardiovasc. Pharmacol. 13 (Suppl. 5), S108.

Watanabe, T., N. Suzuki, N. Shimamoto, M. Fujino and A. Imada, 1991, Contribution of endogenous endothelin to the extension of myocardial infarct size in rats, Circ. Res. 69, 370.

Watanabe, T., Y. Awane, S. Ikeda, S. Fujiwara, K. Kubo, T. Kikuchi, K. Kusumoto, M. Wakimasu and M. Fujino, 1995, Pharmacology of a non-selective ETA and ETB receptor antagonist, TAK-044 and the inhibition of myocardial infarct size in rats, Br. J. Pharmacol. 114, 115.