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Selective endothelin a (ET_A) receptor antagonist (BQ-123) reduces both myocardial infarct size and oxidant injury

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Abstract

Objective: Endothelins (ET) can be considered stress-responsive regulators working in paracrine and autocrine fashion. It has been suggested that elevated levels of ET may be responsible for the low coronary re-flow phenomena. Ischemia–reperfusion (I/R) was shown to stimulate ET release in rat heart; however, the mechanism(s) of this effect has not been clarified. Therefore, this study was focused to investigate the effect of BQ-123, selective ET_A receptor antagonist, on three aspects of myocardial ischemia–reperfusion (MI/R) injury: hemodynamic parameters, infarct size and oxidant–antioxidant status in the absence and presence of ET-1 in an vivo rat model.

Methods and results: To produce MI/R, a branch of the descending left coronary artery was occluded for 30 min followed by 2 h reperfusion. ECG changes, blood pressure (BP), and heart rate (HR) were measured before occlusion and continued both occlusion and reperfusion. Forty rats were randomly assigned to five groups equally: (1) sham-operated rats without coronary ligation, (2) I/R group, (3) I/R + BQ-123-treated group (10 μg/kg/min i.v.), (4) I/R + ET-treated group (25 ng/kg/min i.v.), (5) I/R + ET + BQ-123-treated group. The results are expressed as mean ± S.E.M. In the ET-1 plus I/R group, the ratio between the infarcted area and area at risk 56 ± 1% was significantly higher than I/R group (49 ± 1%). In the BQ-123 group with or without exogenous ET-1 treatment in I/R group, this ratio was significantly lower at 40 ± 2 and 37 ± 1%, respectively. As compared to sham group, I/R increased lipid peroxidation whereas decreased nitric oxide (NO), glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) contents. This decreased antioxidant enzymatic defense could result in aggravated oxidative damage in I/R group rat hearts. ET-1 administration group showed severe oxidative damage. BQ-123 administrations to I/R group with or without ET-1 caused significantly decrease in lipid peroxidation and increased in SOD, CAT activities and NO generation and GSH content when compared with I/R group alone.

Conclusions: The most important finding of the present study is that the ET blockade reduced I/R-induced myocardial injury. The mechanism of this reduction was speculated to be a resistance to ischemic injury in the subcellular levels of the myocardium conferred by a reduction of vascular constriction and improvement of imbalance in the antioxidant status.

Keywords: ET_A receptor antagonist (BQ-123); Endothelin; NO; Reactive oxygen radicals; Rat

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1. Introduction

Endothelin-1 (ET-1), one of the strongest vasoconstructive peptides, is constitutively secreted by vascular cells and cardiomyocytes. This release is increased in various pathophysiological conditions, including myocardial ischemia–reperfusion injury (MI/R) (Liu et al., 1990). In patients with acute myocardial infarction (MI), the plasma ET-1 level was reported to be approximately seven-fold higher (3.43 ± 1.03 pmol) than in healthy volunteers (0.54 ± 0.05) (Lam et al., 1991).

We know now that there are three isoforms of endothelin (ET) named ET-1, ET-2 and ET-3 (Benigni and Remuzzi, 1999). During and following MI/R, the myocardial production and release of ET-1 are stimulated and the coronary constrictor response to ET-1 is enhanced (Battistini and Dussault, 1998). ET-1 is most abundant in cardiovascular system, and has been identified two distinct receptors, classified into two subtypes, ET_A and ET_B (Yamamoto et al., 2005). However, the role of each subtype has not been completely clarified.

The vasoconstrictor effects of ET-1 are mainly due to activation of the ET_A receptor although ET_B receptors also mediate some vasoconstriction (Douglas et al., 1992). Among the ET receptor antagonists BQ-123, selective ET_A receptor antagonist, is used frequently in preclinical research and clinical trials of ET (Masaki, 2004). Recent reports have demonstrated inhibitory effects of BQ-123 on the extension of MI in a dog model (Grover et al., 1993). However, some studies have shown no cardioprotective effects of ET receptor antagonists. For instance, in a study the ET_A receptor antagonist, FRI 139317, showed no beneficial effects in rabbits (McMurdo et al., 1994). Richard et al. (1994) reported that endothelin ET_A and ET_B receptor antagonist, bosentan, did not reduce the I/R-induced myocardial infarct size and concluded that endogenous ET does not contribute to myocyte or coronary endothelial injury in rat model of I/R. It is not clear that whether these beneficial effects are caused by a direct effect on a reduction of the ET-1 vasoconstriction, or some other factors including neutrophil accumulation related to the endothelial dysfunction.

Given the complex ET-1 actions, it is not easy to decide whether increased ET-1 levels are beneficial or deleterious. Some actions suggest that ET-1 is involved in the healing process after MI/R, since it exhibits mitogenic properties, releases pro-inflammatory cytokines, and stimulates proliferation of cardiac fibroblasts and vascular smooth muscle (Noll and Lüscher, 1998). Besides these beneficial effects, ET-1 seems to be an important tool in managing the functional consequences of MI. The extent of ET-1 related MI/R injury seems to several factors, including myocardial blood flow, generation of free radicals, and accumulation of neutrophils. However, the relationship between ET-1 and the cardiac oxidant–antioxidant system in I/R conditions is unclear.

The aim of the present study was designed to test the possible interaction between ET receptor blockade and nitric oxide (NO) production during MI/R. Specifically, the effect of BQ-123, selective ET_A receptor antagonist, was investigated on three aspects of MI/R injury: hemodynamic parameters, infarct size and oxidant–antioxidant status related to the endothelial dysfunction in the absence and presence of ET-1. Also, we focused that whether exogenously applied ET-1 aggravates myocardial infarct size or not.

2. Methods

2.1. Animals and groups

Male Wistar rats aged 10–12 weeks and weighing 200–250 g were placed in temperature ($21 \pm 2\,^{\circ}C$) and humidity ($60 \pm 5\%$) controlled room in which a 12 h:12 h light:dark cycle was maintained. Forty rats were randomly assigned to five groups equally: (1) sham-operated rats without coronary ligation, (2) I/R group, (3) I/R + BQ-123-treated group, (4) I/R + ET-treated group, and (5) I/R + ET + BQ-123-treated group.

For achieve the same experimental protocol conditions in all groups, we infused a comparable volume of the vehicle in sham and I/R groups. In the current study, we used a rat model of in vivo MI/R similar to the one used in our previous studies (Parlakpinar et al., 2005b).

2.2. Ischemia–reperfusion procedure

The rats were anesthetized with urethane 1.2 g/kg administered intraperitoneally (i.p.). The jugular vein and the trachea were cannulated for drug administration and artificial respiration. The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs, about 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately with room air, using a volume of 1.5 ml/100 g body weights at a rate 60 strokes/min to maintain normal PCO2, PO2, and pH parameters. After the pericardium was incised, a gentle pressure on the right side of the rib cage exteriorized the heart. A 6/0 silk suture attached to a 10-mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest, and the animal was allowed to recover for 20 min. Any animal in which this procedure produced arrhythmias or a sustained decrease in mean arterial blood pressure to less than 70 mmHg was discarded. A small plastic snare was threaded through the ligature and placed in contact with the heart. Applying tension to the ligature could then occlude the artery, and

reperfusion was achieved by releasing the tension. The left coronary artery was occluded for 30 min and then reperfused for 120 min more before the experiment was terminated. The duration of I/R and number of rats were chosen on the basis of the previous studies (Liu et al., 1996; Ozer et al., 2004).

2.3. Drug administration

After the rats were anesthetized, the jugular vein was catheterized for drug and vehicle administration. All pharmacological agent treatments began at 10 min before coronary artery occlusion and continued throughout the ischemia period (30 min), totally 40 min for infusion time. We used the most intensively studied antagonist is the ET_A-selective cyclic pentapeptide BQ-123 (Calbiochem, 5 mg Sodium Salt, U.S. and Canada) in a rat model of MI/R. This receptor is well established in a variety of tissues including cardiac membranes and is known to mediate the effects of ET-1. Since Pernow and Wang (1997) suggested that antagonists must be present in the ischemic myocardium at the time of ischemic injury, therefore, we administered BQ-123 before coronary artery occlusion and continued during ischemia. BQ-123 has been reported to completely inhibit ET-1 induced pressure responses in the rat model at doses of 10 μg/kg/min without any effects on heart rate or blood pressure (Garjani et al., 1995).

ET-1 (Sigma–Aldrich Chemie Gmbh, Steinheim, Germany) was dissolved in phosphate buffered saline and injected at a dose of (25 ng/kg/min i.v.). ET-1 dose was selected based on observations of Valentin et al. (1991), who used a similar i.v. dose for constituting of a modest increase in blood pressure of 12% from a baseline of 99 ± 5 mmHg in rats. All experiments in this study were performed in accordance with the guidelines for Animal Research from the National Institutes of Health and were approved by the Committee on Animal Research at Inonu University, Malatya, Turkey.

2.4. Evaluation of hemodynamic parameters

Systemic blood pressure (BP) was monitored from the carotid artery by a Harvard model 50-8952 transducer and displayed on a Harvard Universal penrecorder together with a standard lead-1 electrocardiogram (ECG). ECG changes, mean arterial pressure (MAP), and heart rate (HR) were measured at baseline (i.e. before administration of BQ-123) and at the end of the 30 min period of ischemia, and after 30, 60, and 120 min of reperfusion. HR and MAP were analyzed at selected times to determine the hemodynamic effects associated with coronary occlusion and with drug treatment according to our previous study (Parlakpinar et al., 2005a) which used same experimental protocol.

2.5. Evaluation of tissue death

At the end of the study, the heart was quickly removed and mounted on a Langendorff apparatus where it was flushed with saline at room temperature for 60 s. The coronary branch then re-occluded and zinc–cadmium fluorescent particles (1–10 μm in diameter from Duke Scientific Corb, Palo Alto, CA, USA) were infused into the perfusate to mark the risk zone which is recognized as the area perfused with blood of left coronary artery. The heart was then frozen and a total of four transverse slices, 2 mm in size, from each heart were cut starting from the apex. During this cutting, a 0.5 mm wide full-thickness transverse sample was taken from the second cut from the apex for biochemical investigations according to our previous study (Sahna et al., 2005) and placed into liquid nitrogen and stored at −70 °C until assayed for biochemical analysis (the levels of MDA, NO, SOD and GSH).

The slices were incubated in 1% triphenyl tetrazolium chloride (TTC) in pH 7.4 buffer at 37 °C for 20 min. TTC stains living tissue as deep red color while necrotic tissue is TTC negative and looks tan. The formazan precipitate resulting from the reaction of lactate dehydrogenase in normal and ischemic regions delineated the area at risk from the infarcted tissue. In this procedure, the slices were examined under UV light to visualize the normal area, which appeared bright because of the presence of the fluorescent particles. After we traced this area, each infracted portion within the risk region was also traced onto the same acetate sheet. These acetate tracings were then photocopied and a 100-fold enlarged and the volume of infarct and the risk zone were determined by computerized-planimetry of each tracing and multiplying by the slice thickness. Infarct size was normalized by expressing it as a percentage of the area at risk. The studies were carried out in a blinded fashion so that the investigator conducting the infarct analysis was unaware of the treatment.

2.6. Biochemical determination

The heart sample was homogenized in ice-cold 150 mM KCI for determination of the levels of MDA. The MDA content of homogenates was determined spectrophotometrically by measuring the presence of thiobarbituric acid reactive substances (TBARS) in the heart resulting from a decrease in the polyunsaturated fatty acid content, which serves as a substrate for free radical attacks (Vermeulen and Baldew, 1992). Results are expressed as nmol/g tissue.

One limitation of the present study is that quantitative measurement of NO formation was not performed and related to the effect on infarct size. NO is rapidly oxidized to nitrite (NO_2^-)/nitrate (NO_3^-) (Wennmalm et al., 1992). Since total NO_2^-/NO_3^- originates from several sources other than endothelial NO, determination of plasma NO_2^-/NO_3^- poorly reflects NO production in vivo. Therefore, we measured the concentration of these stable NO oxidative metabolites. Quantitation of NO_2^- and NO_3^- as based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of NO_2^- with a mixture of naphthlethylenediamine and sulfanilamide (Fadillioglu et al., 2003). Results are expressed as μmol/g tissue.

GSH was determined by the spectrophotometric method, which was based on the use of Ellman's reagent (Sahna et al., 2003). Results are expressed as nmol/mg tissue.

CAT activity was determined according to Aebi's method (Aebi, 1974). The principle of the assay is based on the determination of the rate constant (s/k) for hydrogen peroxide (H_2O_2) decomposition at 240 nm. Results were expressed as k (rate constant) g/protein.

SOD enzyme activity determination was based on the production of H_2O_2, from xanthine by xanthine oxidase and reduction of nitroblue tetrazolium as previously described (Aladag et al., 2003). The product evaluated in spectrophotometrically at 560 nm. Results are expressed as U/mg protein.

2.7. Statistics

The results are expressed as mean ± standard error of the mean (S.E.M.). Test of normality was done with Shapiro–Wilk test. Homogeneity of variances was tested by Levene statistic. HR, BP, tissue MDA, NO, SOD, CAT, levels were analyzed by one-way ANOVA. Post hoc comparisons were performed using Tukey's test. All tests were run at an overall 0.05 level of significance.

3. Results

3.1. Hemodynamics in MI/R

The following analyses were performed in 40 rats that survived at the end of 60 min reperfusion. No rats were died and excluded from the analysis because of ventricular fibrillation (frequent reason of I/R related with death) and the other reasons during experimental protocol. Fig. 1 indicates time courses in HR and MAP during I/R in the animals that entered the infarct size study and completed the entire protocol (Table 1).

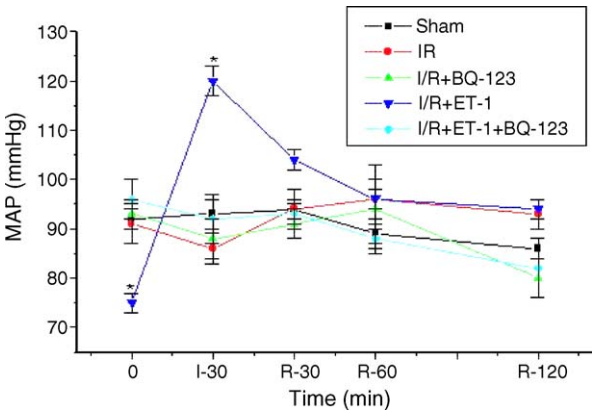

Fig. 1. The graphic of mean arterial pressure (MAP) during ischemia (30 min) and reperfusion (120 min) (I/R) period in the rats pretreated with vehicle (sham and I/R), BQ-123 (selective ET_A receptor antagonist) (10 μg/kg/min), endothelin-1 (ET-1, 20 ng/kg/min) or combined with BQ-123 and ET-1. * Significant difference $p < 0.05$.

There were no significant differences in baseline values for hemodynamic parameters among the all groups. Also, there were no significant differences in changes of HR or MAP during I/R within any of four groups except I/R + ET-1-treated group. I.V. injection of ET-1 (25 ng/kg/min) produced the characteristic biphasic hemodynamic responses described previously (Crino et al., 1997), consisting of an initial transient reduction in MAP (75 mmHg; $n = 8$) followed by a prolonged increase (120 mmHg). The MAP values elicited by ET-1 were significantly attenuated in the presence of a continuous infusion of BQ-123 treatment. However, ET-1 had no effect on HR in the absence or presence of BQ-

Table 1
Summary of mean arterial blood pressure (MAP) and heart rate (HR) in sham, ischemia–reperfusion (I/R), I/R + BQ-1213 (selective ET_A receptor antagonist), I/R + ET-1 and I/R + ET-1 + BQ-1213 groups

	End of stabilization	End of ischemia	Reperfusion (min)		
			30	60	120
MAP (mmHg)					
Sham	92 ± 2	93 ± 3	94 ± 2	89 ± 2	86 ± 2
I/R	91 ± 4	86 ± 3	94 ± 4	96 ± 2	93 ± 3
I/R + BQ-123	93 ± 3	88 ± 4	91 ± 3	94 ± 9	80 ± 4
I/R + ET-1	75* ± 2	120* ± 3	104 ± 2	96 ± 4	94 ± 2
I/R + ET-1 + BQ-123	96 ± 4	92 ± 5	93 ± 2	88 ± 2	82 ± 6
HR (beats/min)					
Sham	324 ± 27	296 ± 18	322 ± 29	320 ± 21	374 ± 20
I/R	307 ± 14	353 ± 18	324 ± 21	309 ± 11	300 ± 13
I/R + BQ-123	319 ± 17	364 ± 13	332 ± 11	320 ± 23	324 ± 19
I/R + ET-1	346 ± 13	369 ± 12	381 ± 22	375 ± 16	332 ± 15
I/R + ET-1 + BQ-123	311 ± 23	320 ± 11	353 ± 9	364 ± 8	349 ± 18

Values are given as mean ± S.E.M, $n = 8$ in each group.
* Significant difference $p < 0.05$.

Table 2
Presents the summary of risk zone, infarct size and infarct size/risk zone ratio data in ischemia–reperfusion (I/R), I/R + BQ-123 (selective ET_A receptor antagonist), I/R + endothelin-1 (ET-1) and I/R + ET-1 + BQ-123 groups

Groups	Risk zone (cm^3)	Infarct size (cm^3)	Infarct size/risk zone (%)
I/R	38 ± 1	18 ± 1	49 ± 1
I/R + BQ-123 (10 μg/kg/min)	38 ± 1	14 ± 1[a]	37 ± 1[a]
I/R + ET-1 (25 ng/kg/min)	37 ± 1	20 ± 1	56 ± 1
I/R + ET-1 + BQ-123	36 ± 1	14 ± 1[a]	40 ± 2[a,b]

Values are given as mean ± S.E.M, $n = 8$ in each group.
[a] Significantly different from I/R group ($p < 0.05$).
[b] Significant difference resulted from BQ-123-treated group ($p < 0.05$).

123 between baseline and reperfusion. Clearly BQ-123 concentration is sufficient to block the vasoconstrictor effects caused by ET-1 in the coronary vasculature of the rat. In the current experiments BQ-123 (10 μg/kg/min) markedly inhibited the pressor response to ET-1, in agreement with previous study (Valentin et al., 1991).

3.2. Myocardial necrosis in MI/R

Table 2 summarizes the area at risk, which is a major predictor of infarct size in models of regional ischemia, and infarct size data for four groups except sham. Examination of the data for percentage of the left ventricle at risk of infarction shows that no differences existed between I/R and treated groups, indicating that all groups had the same potential for ischemic damage as a result of coronary artery occlusion. Likewise, the left ventricular weights of these animals were not significantly different (data not shown). In the ET-1 group, the ratio between the infarcted area and area at risk 56 ± 1% was significantly higher than I/R group (49 ± 1%). In the BQ-123 group with or without exogenous ET-1 treatment in I/R group, this ratio was significantly lower at 40 ± 2 and 37 ± 1%, respectively. The percentage of the risk area that actually proceeded to infarction was found to be significantly smaller in the rats that received BQ-123 than in those that were untreated (I/R) and ET-1 applied alone groups. When ET-1 was injected in the presence of the ET_A antagonist, the marked inhibitory effects on cardiovascular function were not observed when compared with ET-1 treatment alone (Fig. 2).

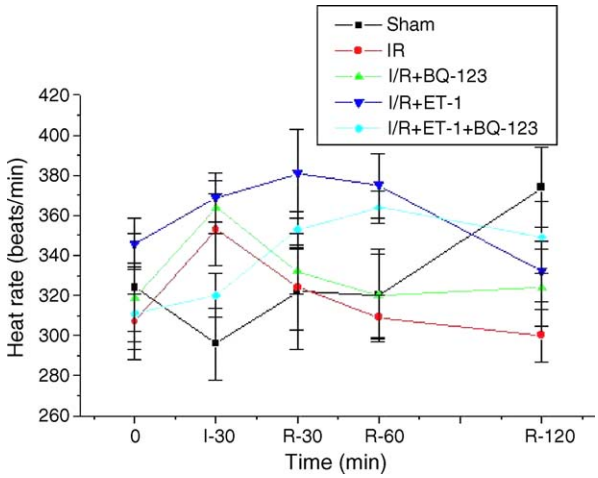

Fig. 2. The graphic of heart rate (HR) during ischemia (30 min) and reperfusion (120 min) (I/R) period in the rats pretreated with vehicle (sham and I/R), BQ-123 (selective ET_A receptor antagonist) (10 μg/kg/min), endothelin-1 (ET-1, 20 ng/kg/min) or combined with BQ-123 and ET-1.

3.3. Biochemical changes in MI/R

The biochemical results have shown in Table 3. Briefly, I/R-induced cardiotoxicity, manifested by a significantly increased tissue MDA, the end product of lipid peroxidation, while SOD, CAT activities and NO production and GSH content significantly decreased

Table 3
The effects of ischemia–reperfusion (I/R) or BQ-123 (selective ET_A-receptor antagonist) administration on heart tissue enzymes in ischemia–reperfused or sham-operated rats with or without endothelin-1 (ET-1) treatment

Parameters	Sham	I/R	I/R + BQ-123	I/R + ET-1	I/R + ET-1 + BQ-123
MDA (nmol/g tissue)	103.1 ± 4.5	206.7 ± 13.8[a]	119.4 ± 10.8[b]	305.2 ± 17.5[a,b]	152.8 ± 16.1[b,c]
NO (μmol/g tissue)	145.8 ± 35.7	82.6 ± 3.05[a]	130.6 ± 6.3[b]	50.8 ± 3.7[a,b]	106.9 ± 3.9[a,b,c]
GSH (nmol/g tissue)	3081.1 ± 16.9	1427.1 ± 12.1[a]	2529.5 ± 17.0[b]	1538.6 ± 36.7[a]	2232.9 ± 5.3[a,b,c]
CAT (k/g protein)	17.1 ± 1.4	10.0 ± 0.3[a]	15.3 ± 0.6[b]	5.46 ± 0.8[a,b]	12.4 ± 0.3[a,c]
SOD (U/mg protein)	2.7 ± 0.2	1.8 ± 0.06[a]	2.5 ± 0.2[b]	1.12 ± 0.08[a,b]	2.1 ± 0.1[a,c]

Values were expressed as mean ± S.E.M.
[a] $p < 0.05$ vs. sham group.
[b] $p < 0.05$ vs. I/R group.
[c] $p < 0.05$ vs. I/R + ET-1 group.

when compared to sham group. The aggravated oxidative damage in I/R group rat hearts can cause the decreased antioxidant enzymatic defense. ET-1 administration group also showed severe oxidative damage.

BQ-123 administration to I/R group with or without ET-1 caused significantly decrease in lipid peroxidation and increase in SOD, CAT activities and NO generation and GSH content when compared with I/R group alone.

4. Discussion

The main findings of the current study were as follows: (1) the selective ET_A receptor antagonist, BQ-123, prevented the coronary vasoconstrictor effect of both endogenous and exogenous ET-1; (2) BQ-123 reduced the extent of myocardial infarct size triggered by I/R and I/R + ET-1; (3) BQ-123 showed protective effects on the enzymatic defense system and lipid peroxidation when compared to the I/R group and the ET-1-treated group; (4) BQ-123 treatment did not change hemodynamic parameters (HR and MAP).

Parallel to our findings Pollock and Opgenorth (1993) reported that the systemic pressor effect of ET-1 in rat was abolished by BQ-123. BQ-123 treatment in the I/R group also no significant changes in the HR or MAP. Likewise, Grover et al. (1993) reported that BQ-123 administration during a 90 min period of coronary arterial occlusion did not change the hemodynamic parameters whereas decreased the infarct size.

In the current study, we demonstrated that a specific antagonist of the ET_A receptor could reduce the myocardial injury that occurs in a rat model of MI/R. On account of fact that, Basso et al. (2002) reported that selective ET_A receptor blockade improved endothelial function while the mixed ET_A–ET_B blockade did not, therefore, we used in this study specific ET_A receptor antagonist, which is the major form of human cardiomyocytes (Cernacek et al., 2003).

Results of studies investigating I/R injury related to ET receptor blockade in the heart are conflicting. Later studies reported favorable effects of selective ET_A receptor blockade in myocardial function including infarct size during I/R (Cernacek et al., 2001). Yet other studies did not observe any effects of ET receptor blockade on myocardial function (Basso et al., 2002). In line with these findings Kojima et al. (1995) reported that the mixed ET_A/ET_B receptor antagonist TAK-044 reduces infarct size in rats, rabbits and dog when administered either before or after induction of AMI. In a study, BQ-123 was given 15 min before and continuously throughout I/R, reduced by 40% myocardial infarct size in dogs subjected to 90 min ischemia and 5 h reperfusion. Additionally, BQ-123 also had no effect on regional myocardial blood flow in ischemic and nonischemic tissue (Grover et al., 1993).

One possibility is that these antagonists induce vasodilatation and prevent development of the no-reflow phenomenon. However, the mechanism of this phenomenon is not fully demonstrated, several factors may contribute including oedema, and stiffening of the myocytes, endothelial cell swelling, microvascular spasm and plugging of the vessels with inflammatory cells (Hansen, 1995). A second possibility is interaction with neutrophils. Activated neutrophils are assumed to be important contributors to tissue injury following reperfusion of the ischemic myocardium. Based on this relationship, it has shown that ET-1 is involved in the activation of neutrophils and may enhance the production of superoxide anion (Ishiada et al., 1990). It is well established post-AMI ET-1 levels in the scar are manyfold higher than in myocardium. These results strongly suggest that ET-1, a recognized fibrogenic factor, plays a crucial role in the stabilization of the necrotic area and in the healing of the scar (Cernacek et al., 2003). A third possible mechanism for the observed cardioprotective effect of ET antagonists may be inhibition of direct effects of ET-1 on the myocardial cell. A four favorable mechanism is that ET receptor blockade attenuates activity of renin-angiotensin and sympathetic nervous systems (Webb et al., 1997).

In contrast, in a study i.v. administration of BQ-123 did not affect infarct size in the dog following 90 min of coronary occlusion and 4 h reperfusion (Karuse et al., 1994). In another study, in dogs subjected to 40 min ischemia followed by 4 h reperfusion, administration of FRI 139317, selective the other ET_A receptor antagonist did not alter infarct size (Erikson and Velasco, 1996).

One possible explanation for the increased vasoconstriction seen with ET treatment in our study may be through a reduced ability to secrete NO in ischemic coronary arteries. Exogenously administered ET-1 has been demonstrated to increase peripheral resistance and blood pressure, presumably due to the release of vasodilatory compounds such as NO (Kramer et al., 1997). These data contradict an idea that the endothelial dysfunction was due to the destruction of the endothelium derived NO by ROS. NO plays a crucial role not only in the regulation of vascular tones but also in the prevention of platelet and leukocyte adherence and the inhibition of superoxide accumulation (Palmer et al., 1988).

In the current study, exogenously applied ET-1 also enhanced I/R-induced oxidant injury and worsened the cardiac dysfunction observed after reperfusion, thereby suggesting that post-ischemic dysfunction results at least

in part from ET-1 induced excessive MDA release and reduced GSH, SOD, CAT levels, as well as peptide-induced coronary vasoconstriction. In addition, the above detrimental ET-1 actions were completely suppressed by BQ-123 treatment, which indicates that both endogen and exogenous ET-1-induced actions are also mediated exclusively by ET_A receptor. Furthermore, reduced levels of NO were reversed by BQ-123 concomitant treatment with or without ET-1 suggest that both I/R and ET-1 reduced the level of NO thereby mediated cardiotoxicity. Parallel to our this notion, recently Ganon et al. (1998) reported that the dual ET_A/ET_B receptor antagonist bosentan preserved endothelial and cardiac contractile function during I/R via a mechanism dependent on endothelial NO production. Additionally, recently Hong et al. (2003) reported that pretreatment with PD 156707 (ET_A receptor antagonist) improved the ischemic isolated heart function, enhanced SOD activity and decreased MDA content in the ischemic myocardium.

It is well documented that I/R results in impaired endothelium-dependent vasodilatation, which is the result of reduced NO formation or rapid inactivation of NO by ROS (Lefer et al., 1997). ROS have been implicated in the mechanism various forms of MI/R injury (ref apo), including the post ischemic endothelial dysfunction (Beresewicz et al., 1998). Current research has raised the exciting prospect that endothelial dysfunction is potentially reversible (Bhagat, 1998). It is well documented that coronary endothelial dysfunction could be prevented by SOD (Tsao et al., 1990). Regarding the ROS-induced endothelial dysfunction, we demonstrated those amounts of SOD, CAT enzymes and NO, GSH, MDA content.

ET-1 is linked to the dysfunction of the L-arginine/NO pathway since ET_A-selective but not combined ET blockade improves endothelial function, independent of blood pressure. Thus, selective inhibition of ET_A receptors improves the endothelial L-arginine/NO pathway (Lüscher and Barton, 2000). The activation of endothelial ET_B receptors also stimulates the release of NO, prevents cell death. It has also been shown that ET_B receptor stimulation does not any proischemic effect. Therefore, related to mixed ET receptor antagonist attenuated myocardial infarct size strongly indicated that this detrimental effect mediated by ET_A receptor. We conclude that ET-1 promotes ROS overflow and induces ET_A-mediated coronary vasoconstriction, both of which lead to subsequent cardiac dysfunction in the heart. Due to endothelial dysfunction the exaggeration of neutrophil accumulation may lead to over production of ROS from heart.

Taken together, the results of the current study would suggest that activation of the cardiac ET system due to endothelial dysfunction plays a major role in occurring expansion of myocardial infarct size ratio. In summary, the results of this study implicate a following sequence of events in the mechanism of the post-ischemic endothelial dysfunction: I/R, ET-induced neutrophil accumulation, reduced NO production and increased ROS due to failed antioxidative defense mechanisms and/or some of its toxic metabolite, damage to the endothelium. Finally, we demonstrated here that both endogenous and exogenous ET-1 play a major role in the extension of infarct size in rats correlated reduced NO production and antioxidative defense mechanism. Further investigations with longer animal models, dosage determination, and more detailed mechanism are needed to concerning the exact protective mechanism of BQ-123.

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