Endothelin Receptor Blockade with Tezosentan Ameliorates Myocardial Injury Induced by Abdominal Aortic Ischemia-Reperfusion

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Endothelin is both a potent vasoconstrictor and an important mediator of ischemia-reperfusion (IR) injury. Therefore, the role of endothelin receptor antagonism in IR-induced tissue injury carries great interest. Here, we examined the effect of tezosentan, a nonselective antagonist for endothelin receptors, on myocardial injury induced by abdominal aortic IR, which represents a model of the IR injury in distant organs frequently occurred after vascular surgery. Thirty-two Wistar rats were randomized into four groups (n = 8) as follows: control (sham laparotomy), aortic IR (120 min ischemia and 120 min reperfusion), aortic IR + tezosentan (10 mg/kg intravenous injection before ischemia plus continuous intravenous infusion of 1 mg/kg/hr during the IR injury), and control + tezosentan. Biochemical analysis showed that aortic IR significantly increased (p < 0.05 vs control) the plasma levels of troponin-I, interleukin-6 and tumor necrosis factor-alpha, and the myocardial tissue levels of malondialdehyde, superoxide dismutase and catalase, whereas tezosentan significantly decreased these same factors (p < 0.05 vs aortic IR). Histological evaluation also showed that aortic IR significantly increased (p < 0.05 vs control) myocardial disorganization, myofiber swelling and myofiber eosinophilia in myocardial tissue samples, whereas tezosentan significantly decreased these factors (p < 0.05 vs aortic IR). These results indicate that tezosentan has protective effects against myocardial injury induced by abdominal aortic IR in rats. We propose that the mechanisms underlying this protective effect of tezosentan involves the reduction of oxidative stress and subsequent lipid peroxidation, the inhibition of systemic inflammatory response, and acting cytoprotective on myocytes after aortic IR. ——— Abdominal aortic surgery; reperfusion injury; reactive oxygen species; acute-phase reaction; endothelin-1.

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Abdominal aortic aneurysm (AAA) and aortoiliac occlusive disease are common diseases of vascular surgery, and both generally require open laparotomy, bowel manipulation, aortic cross-clamping, and prosthetic graft interposition (Galle et al. 2000). Abdominal aortic cross-clamping leads to an ischemic insult to the lower extremities, and subsequent reperfusion results in excessive generation of reactive oxygen species (ROS), leading to oxidative stress (Gelman 1995). Oxidative stress promotes induction of an inflammatory response due to the increased production of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-2, IL-6 and interferon (Seekamp et al. 1993; Carden and Granger 2000; Papaharalambus and Griendling 2007). Both oxidative stress and inflammatory response often result in ischemia-reperfusion (IR) injury in distant organs (Bown et al. 2001). Such injuries are most frequently observed in the lung, kidney, and cardiovascular system, and can result in the development of systemic inflammatory response syndrome and multiple organ dysfunction syndrome, which taken together account for 30-40% of the mortality in tertiary referral intensive care units (Carden and Granger 2000; Hafez et al. 2000). Abdominal aortic IR-induced myocardial injury has been shown in experimental studies (Grünenfelder et al. 2002; Kiris et al. 2007).

Myocardial injury after abdominal aortic surgery, detected by a rise in serum cardiac troponin (Tn), is common and associated with decreased survival (Kim et al. 2002; Ali et al. 2008). Significant ST segment depression on ECG as a sign of myocardial injury has been shown in up to 40% of patients undergoing elective AAA surgery (Rapp et al. 1999). Myocardial injury, detected by rises in TnI, has been described in 25-47% of patients undergoing elective AAA surgery (Ali et al. 2008).

The ROS-induced oxidative stress and inflammatory response in cardiac and vascular myocytes has been linked with cardiovascular tissue injury (Valko et al. 2007). Oxidative stress results in sarcoplasmic reticular dysfunction and increased membrane permeability, so this leads to subsequent intracellular Ca^{2+} overload, myocardial cell damage, and cardiac dysfunction (Dhalla et al. 2000). Inflammatory response induces neutrophil activation as well as generalized leukocyte and endothelial adhesion molecule expression, which promotes the opportunities for leukocyte-endothelial cell interaction, and finally IR-induced remote organ injury is enhanced, along with the effect of oxidative stress (Carden and Granger 2000).

In the course of IR injury, many mediators play an important role in the pathogenesis of distant organ injury. Endothelin (ET)-1, one of the most contributed mediators during IR injury, increases in the few hours of reperfusion (Herbert et al. 2001). The ETs are a family of 21 amino acid peptides with powerful vasoconstrictive properties, and evidences indicate that they also have proinflammatory actions, such as superoxide production of neutrophils, cytokine release from macrophages or monocytes, and upregulation of adhesion molecules, leading to an aggravation of microcirculatory and tissue damage (Mitsuoka et al. 1999; Rossi et al. 2004; Uhlmann et al. 2005). ET-1, which is probably the most important of the ETs, is produced mostly by the vascular endothelium and acts on different subtypes of receptors. ET receptor type A and ET receptor type B are located on vascular smooth muscle cells and mediate contraction. ET receptor type B, located on the endothelium, mediates vasodilation through the release of nitric oxide and prostacyclin (Rossi et al. 2004; Dammanahalli and Sun 2008). Oxidative stress and ROS increase ET-1 generation (Carden and Granger 2000; Dammanahalli and Sun 2008). Endothelin-1 stimulates leukocyte rolling and adhesion, and enhanced inflammatory reaction (Muller et al. 2000; Callera et al. 2003). Also, ET-1 induces post-ischemic superoxide generation, and thus ET-1-mediated superoxide generation causes post-ischemic endothelial dysfunction and endothelial glycocalyx disruption in the cardiovascular system, which contributes to microvascular injury and resulting myocardial damage (Duda et al. 2006; Singh et al. 2006). Endothelin receptor antagonists afford endothelial protection by preventing protein kinase C and NADPH oxidase
activation, and related overproduction of toxic oxidants in the post-ischemic guinea-pig hearts (Maczewski and Beresewicz 2000; Kurzelewski et al. 2002; Duda et al. 2006). The use of endothelin receptors antagonists provides beneficial effects in terms of the reduction of infarct size and improved recovery of myocardial performance and coronary flow after IR (Climent et al. 2006).

Tezosentan is a potent and highly watersoluble ET receptor antagonist specifically designed for parenteral use (Clozel et al. 1999). It has a nonselective antagonistic effect on both ET-A and ET-B receptors. The short half-life of tezosentan should allow its effects to plateau rapidly, consequently permitting its dosage to be easily adjusted (Chin et al. 2001). Its specificity decreases the potential for side effects. These characteristics make tezosentan a particularly attractive ET antagonist (Clozel et al. 2002). Because ET is believed to play a role in the pathophysiology of IR injury, ET antagonists were used effectively to ameliorate IR injury-related organ damage such as skeletal-muscle (Herbert et al. 2001), bowel (Mitsuoka et al. 1999), liver (Uhlmann et al. 2005), heart (Maczewski and Beresewicz 2000; Clozel et al. 2002; Kurzelewski et al. 2002; Climent et al. 2006; Duda et al. 2006; Singh et al. 2006), and kidney (Wilhelm et al. 2001).

In this study, we sought to determine whether endothelin receptor antagonism, represented by tezosentan, ameliorates myocardial damage induced by abdominal aortic IR in rats. To determine this, the present study was designed to assess (i) the heart tissue levels of malondialdehyde (MDA), superoxide dismutase, catalase and myeloperoxidase, (ii) the plasma concentrations of TNF-α, IL-6, ET-1 and TnI and (iii) the histological changes in the hearts of rats specimens subjected to infrarenal abdominal aortic IR.

**Material and Methods**

**Animals**

Thirty-two Wistar rats, of both sexes and weighing 200-250 g, were used for the experiment. The experimental protocols were approved by the Animal Ethics Committee of Suleyman Demirel University Medical School (April 2, 2007, No 03/23). The rats were acquired from the university vivarium sources and were housed in individual cages in a temperature and light-dark cycle-controlled environment with free access to food and water. All rats received humane care, in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, as prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

**Experimental groups**

The rats were randomly allocated into one of the four experimental groups (n = 8); control, aortic IR, aortic IR + tezosentan and control + tezosentan. The control group (sham laparotomy) underwent midline laparotomy and dissection of the infrarenal abdominal aorta (IAA) without occlusion. The aortic IR group underwent 120 min of aortic ischemia and 120 min of reperfusion. The aortic IR + tezosentan group underwent the same aortic ischemia and reperfusion periods as in the aortic IR group and received tezosentan. The control + tezosentan group underwent laparotomy and dissection of IAA without occlusion, and received tezosentan. The rats in the control and aortic IR groups received saline solution. Tezosentan (Actelion Pharmaceuticals Ltd, Switzerland) was diluted in saline and given both as a bolus intravenous injection of 10 mg/kg before aortic ischemia and as a continuous intravenous infusion of 1 mg/kg/hr during 120 min ischemia and 120 min reperfusion in the aortic IR + tezosentan group. In the control + tezosentan group, the same dose of tezosentan was used in the same time period as in the aortic IR + tezosentan group. The dose of tezosentan and timing of infusion were decided according to the literature (Chin et al. 2001, Clozel et al. 2002).

**Aortic ischemia-reperfusion**

The rats were anesthetized with ketamine hydrochloride (Ketalar®, Eczacibaşı, Istanbul, Turkey, 50 mg/kg intramuscular), and anesthesia was maintained with supplementary intramuscular injections of ketamine hydrochloride (Okutan et al. 2004; Kiris et al. 2007). The rats were placed supine under a heating lamp. The skin was aseptically prepared and a midline laparotomy was done. Ten ml of warm normal saline were instilled into the peritoneal cavity to help maintain fluid balance.
The abdominal aorta was exposed by gently deflecting the loops of intestine to the left with moist gauze swabs. An atraumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int., St. Paul, MN, USA) was placed across the IAA. The abdomen was then closed and the wound was covered with plastic wrap to minimize heat and fluid losses. After 120 min, the microvascular clamp on the IAA was removed and the lower limbs were reperfused for 120 min. During reperfusion, aortic vessel patency was confirmed by the reappearance of satisfactory pulsation on the distal aorta. At the end of reperfusion, a median sternotomy was done and blood samples were drawn from the right ventricles of all rats for biochemical analysis. All rats were killed under anesthesia and the hearts were carefully removed en bloc from the chest. The specimens were divided into two pieces: one piece stored in 10% formaldehyde in phosphate-buffered saline solution for histopathological examination, and the other was stored at −80°C for biochemical assays.

Biochemical Analysis

Frozen tissue samples of the rat hearts were weighed and homogenized (Ultra Turrax T25, Janke & Kunkel GmbH & Co., KG, Staufen, Germany) (1:10, w/v) in 100 mmol/L phosphate buffer (pH 7.4) containing 0.05% sodium azide in an ice bath. The homogenates were sonicated (Bandelin Sonoplus UW 2070, Berlin, Germany) for 30 sec and centrifuged at 5000 g for 10 min. The supernatants were frozen at −78°C in aliquots until used for biochemical assays. The protein content of the supernatants was determined by the Lowry method (Lowry et al. 1951). In tissue samples, the levels of MDA, superoxide dismutase, catalase and myeloperoxidase were measured.

The blood samples were kept at room temperature for 30 min, and then were separated into plasma and erythrocytes by centrifugation at 4000 g for 10 min. All samples were kept at −80°C until the date of analysis. In the blood samples, the plasma levels of TNF-α, IL-6, ET-1 and TnI were measured.

MDA, superoxide dismutase, catalase and myeloperoxidase

The MDA level in rat heart tissue was assayed spectrophotometrically by a commercial kit (Bioxytech® MDA-586™, OxisResearch™, CA, USA) according to the manufacturer’s instructions. The superoxide dismutase and catalase activities of rat heart tissue were determined spectrophotometrically at 450 nm and 540 nm, using Cayman Chemical Company (Michigan, USA) assay kits. The myeloperoxidase activity of rat heart tissue was determined using BioCheck’s ELISA kits (BioCheck, Inc. CA, USA) according to the manufacturer’s instructions.

TNF-α, IL-6, ET-1 and TnI

Plasma concentration of TNF-α was measured with a commercial kit (recombinant rat TNF-α kit, BioSource™, Invitrogen Corporation, CA, USA) using an enzyme-linked immunosorbent assay (ELISA) method in an absorbance microplate reader (ELx808™, Biotek Instruments Inc, Vermont, USA). Plasma concentration of IL-6 was measured with a commercial kit (recombinant rat IL-6 kit, BioSource™, Invitrogen Corporation, CA, USA) using an ELISA method in an absorbance microplate reader (ELx808™, Biotek Instruments Inc, Vermont, USA). Plasma concentration ET-1 was measured by an enzyme immunoassay method using a commercial kit (rat big endothelin-1 EIA kit, Assay Desings Inc, Michigan, USA). Plasma concentration of TnI was measured by a chemiluminescent immunometric assay method (Elecsys 2010, Tokyo, Japan).

Histopathological Examination

The heart was removed from rats at the end of the experimental period and was fixed by immersion in 10% formaldehyde in phosphate-buffered saline solution. Following fixation, the heart was trimmed and cut to provide a transverse section through the middle of the ventricles and longitudinal sections of the apex and base of the heart, as Isaacs described (1998). After dehydration using graded ethanol, the tissue was embedded in paraffin and cut into fine (3-μm) sections using a microtome blade. Sections were mounted on glass slides and dried at 70°C. Tissue sections were then deparaffinized with xylene for 20 min, dehydrated with 96% alcohol for 20 min, and counterstained with hematoxylin and eosin. Sections were viewed under a light microscope and evaluated in a blinded manner by a pathologist. Heart specimens were evaluated in terms of myocardial disorganization, myofiber swelling and myofiber eosinophilia. Histological changes were graded as follows: grade 0, no changes; grade 1, focal, mild changes; grade 2, multifocal, intermediate changes; and grade 3, prominent, extensive changes.
Statistical Analysis

Data are presented as means ± standard deviation (S.D.). A computer program (SPSS version 16.0, SPSS Inc. Chicago, IL, USA) was used for statistical analysis. During statistical analysis of the biochemical data, the differences between the groups were determined by one-way ANOVA followed by a post hoc Tukey’s honestly significant difference test. A P-value of less than 0.05 was considered to indicate significance. During statistical analysis of the histological data, the differences between the groups were determined by the Kruskal-Wallis and Mann-Whitney U tests. A P-value of less than 0.05 was considered to indicate significance.

RESULTS

Biochemical analysis

The results of the biochemical analysis are shown in Table 1. Aortic IR significantly increased the plasma levels of TNF-α, IL-6 and TnI (p < 0.05 vs control group). Tezosentan treatment caused a significant decrease in the plasma levels of TNF-α, IL-6 and TnI (p < 0.05 vs. aortic IR). Aortic IR significantly increased plasma ET-1 level (p < 0.05 vs control and control + tezosentan groups). Tezosentan treatment further significantly increased the plasma level of ET-1 in the aortic IR + tezosentan group (p < 0.05 vs other groups). Administration of tezosentan to control rats did not affect the level of any biochemical parameter (p > 0.05 vs control group).

Aortic IR significantly increased the heart tissue levels of MDA, superoxide dismutase and catalase (p < 0.05 vs control group). Tezosentan treatment caused a significant decrease in the heart tissue levels of MDA, superoxide dismutase and catalase (p < 0.05 vs aortic IR group). In the aortic IR group, the tissue level of myeloperoxidase activity was higher than that in the control group, but the difference was not statistically significant (p = 0.12). In the aortic IR + tezosentan group, the tissue level of myeloperoxidase activity was lower than that in the aortic IR group but the difference was not statistically significant (p = 0.95).

Histopathological findings

The results of the histological evaluation with Hematoxylin-Eosin staining are shown in Table 2 and Fig. 1. Histological evaluation with Hematoxylin-Eosin staining showed that myocardial disorganization, myofiber swelling and myofiber eosinophilia were significantly higher in the aortic IR group than in the other groups (p < 0.05, Table 2), (Fig. 1B). In the aortic IR + tezosentan group.

Table 1. Results of the biochemical analysis.

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<th>The levels in plasma</th>
<th>The levels in myocardial tissue</th>
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<tr>
<td></td>
<td>TNF-α (pg/ml)</td>
<td>IL-6 (pg/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>29.23 ± 17.94</td>
<td>89.40 ± 22.29</td>
</tr>
<tr>
<td>Aortic IR</td>
<td>123.78 ± 74.6</td>
<td>183.42 ± 34.97</td>
</tr>
<tr>
<td>Aortic IR +</td>
<td>73.51 ± 24.6a</td>
<td>119.23 ± 38.98b</td>
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<tr>
<td>tezosentan</td>
<td>52.60 ± 23.72</td>
<td>92.13 ± 22.41</td>
</tr>
</tbody>
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Tn I, Troponin I; TNF-α, Tumour necrosis factor alpha; IL, Interleukin; MDA, Malondialdehyde; SOD, Superoxide dismutase; CAT, Catalase; MPO, Myeloperoxidase.

*p < 0.05 compared to other groups.

b<p < 0.05 compared to aortic IR group.

*c<p < 0.05 compared to other groups.

Data are expressed as the mean ± S.D.
group, tezosentan significantly decreased myocardial disorganization, myofiber swelling, and myofiber eosinophilia ($p < 0.05$ vs aortic IR) (Table 2; Fig. 1C). There were no histological changes in the control and control + tezosentan groups (Table 2; Fig. 1A and 1D). There was no histopathological change in terms of inflammatory cell infiltration in all the groups.

**DISCUSSION**

The data obtained from the present study indicate that tezosentan, a mixed endothelin-A and -B receptor antagonist, reduces the myocardial damage associated with abdominal aortic IR in
rats. The main findings of the present study supporting this conclusion can be summarized as follows: (i) biochemical analyses showed that tezosentan reduced oxidative stress, systemic inflammatory response, and TnI levels and, (ii) histological evaluation showed that tezosentan attenuated the morphological changes associated with myocardial injury induced by aortic IR. These findings support the importance of the ET system and protective effect of tezosentan in aortic IR-induced myocardial injury.

During abdominal aortic IR, release of ET is stimulated and the concentration of ET-1 is increased in plasma. This significant increase indicates the important role of ET-1 in abdominal aortic IR injury (Herbert et al. 2001). The activation of the ET system may contribute to myocardial damage in abdominal aortic IR through several different mechanisms. ET has a vasoconstrictor effect, and arteriolar vasoconstriction is considered to contribute to the no-reflow phenomenon (Herbert et al. 2001). ET also activates inflammatory and thrombotic cascades; both contribute to IR-induced distant organ injury (Muller et al. 2000).

Production of ROS by abdominal aortic IR injury leads to peroxidation of membrane phospholipids in the myocardium (Kiris et al. 2007). Lipid peroxidation is considered a major mechanism of tissue damage, and MDA is a good indicator of the rate of this reaction (Gao et al. 2002). Okutan et al. (2004) demonstrated that aortic IR increased tissue MDA level in the lungs and further stated that MDA is a good indicator of aortic IR-induced distant organ injury. In the present study, increased myocardial tissue MDA levels were significantly decreased by tezosentan treatment. These findings indicate that tezosentan decreased lipid peroxidation and prevented membrane damage and cell death in the myocardium during abdominal aortic IR injury.

Major reactive oxygen species, which are formed as a result of aortic IR, are superoxide (O$_2^-$) radicals, hydrogen peroxide (H$_2$O$_2$), and hydroxyl (OH) radicals (Valko et al. 2007). O$_2^-$ is produced by xanthine oxidase, the enzyme catalyzing the hypoxanthine and xanthine metabolism. The enzyme superoxide dismutase rapidly reacts with O$_2^-$ and dismutates it to less reactive H$_2$O$_2$ (Csonka et al. 2000). H$_2$O$_2$ is further converted to H$_2$O and O$_2$ by the enzymes catalase and glutathione peroxidase, and this reaction prevents the formation of the highly reactive OH$^-$ (Zhu et al. 2000). Many studies have demonstrated that in the event of IR, the tissue levels of superoxide dismutase and/or catalase increase so as protect cells from the detrimental effects of ROS (Bolcal et al. 2007; Kiris et al. 2007; Topcu et al. 2007). In our study, we found that aortic IR increased whereas ET receptor antagonism with tezosentan reduced the levels of superoxide dismutase and catalase in the heart tissue. This finding may indicate that tezosentan significantly reduced aortic IR-induced oxidative stress through its antioxidant and cell protective effects.

Many studies have demonstrated that aortic IR injury leads to neutrophil activation and subsequent infiltration into distant organs. Myeloperoxidase, secreted by activated neutrophils, is employed as a sensitive index of neutrophil accumulation and activation in the tissue, and correlates with the intensity of IR injury (Seekamp et al. 1993; Herbert et al. 2001; Kiris et al. 2007). Based on the results of these experimental studies, we measured tissue myeloperoxidase activity, and, in addition, compared this level with the results of histopathological evaluation in terms of leukocyte infiltration in hearts subjected to aortic IR. In our study, significant leukocyte infiltration into myocardial tissue as shown by tissue levels of myeloperoxidase and histopathological examination of the heart tissue was not found after aortic IR. We speculate that the 120-minute reperfusion period may have been sufficient to create significant oxidative stress and systemic inflammatory response but insufficient to elicit significant leukocyte infiltration into the myocardium.

ET-1 is a significant trigger of inflammatory pathways (Uhlmann et al. 2005). ET-1 has been previously shown to induce IL-6 production in endothelial cells, to activate TNF-α, granulocyte-macrophage colony-stimulating factor, IL-1β, IL-6 and IL-8 production in monocytes (Cunningham 1997). TNF-α, like several other
Cytokines, has been shown to stimulate ET-1 secretion, suggesting regulatory feedback between ET-1, TNF-α, and other cytokines (Weitzberg et al. 1996; Uhlmann et al. 2005). In the present study we found that the plasma levels of ET-1, TNF-α and IL-6 simultaneously increased after aortic IR, a finding that indicates the interdependency between these proinflammatory cytokines. On the other hand, tezosentan treatment decreased the levels of both TNF-α and IL-6 in spite of further increased levels of ET-1. Although the latter finding may seem to contrast with the former data, tezosentan’s anti-inflammatory effect has also been reported in some experimental studies. Uhlmann et al. (2005) recently demonstrated that treatment with an ET-A receptor antagonist reduced the expression of prepro-ET-1, pro-TNF-α, and pro-IL-6 after experimental liver IR. Urbanowicz et al. (2004) found that tezosentan decreased the plasma TNF-α level with tezosentan in that setting remained unclear. Therefore, we may remark that ET receptor antagonism with tezosentan mitigates the activation of inflammatory pathways during abdominal aortic IR injury.

Tezosentan, a dual ET receptor antagonist, was proved to be effective in the treatment of IR injury-induced myocardial, pulmonary, renal and intestinal injury (Wilhelm et al. 2001; Clozel et al. 2002; Kurzelewski et al. 2002; Lugowska-Umer et al. 2008). Mitsuoka et al. (1999) demonstrated that ET receptor antagonism by TAK-044 successfully attenuated the development of pulmonary edema in rats subjected to intestinal IR through to preventive effect on the activation of the inflammatory process. Weitzberg et al. (1996) reported that bosentan, a nonselective ET receptor antagonist, increased cardiac output by means of increased coronary circulation and improved cardiac contractility in a porcine endotoxin shock model which activates an inflammatory mechanism similar to IR injury. They stated that another explanation for the increase in cardiac output could be the reduction in afterload. They also suggested that ET receptor antagonism led to improved oxygen delivery to the tissue by increasing cardiac output and regional blood flow to organs in this model.

Singh et al. (2006) remarked that the decrease in both arterial pressure and myocardial oxygen demand might translate into a beneficial effect on endothelin receptor antagonists. They stated that the inactivation of nitric oxide by excessive generation of ROS resulting from IR injury leads to the inactivation of nitric oxide-induced vasodilatation and increased arterial pressure. They concluded that the reduction effect of tezosentan on the generation of ROS may also diminish the inactivation of nitric oxide-induced vasodilatation and may contribute to its cardioprotective effect, independent of the receptor blockade by tezosentan.

Since TnI was proved to be a suitable biomarker of cardiac damage in rats (York et al. 2007), TnI was employed as an indicator of myocardial injury induced by aortic IR in this study. In the present study we observed a significant increase in serum TnI level in the aortic IR group. This result was consistent with the increased myocardial tissue levels of MDA, antioxidant enzymes and increased serum levels of inflammatory markers in the aortic IR groups. In the aortic IR + tezosentan group, rats had a significantly decreased serum TnI level compared to the aortic IR group. Decreased serum TnI levels correlated with decreased MDA, antioxidant enzymes and inflammatory markers in the aortic IR + tezosentan group. Our results are consistent with those of Singh et al. (2006), who reported the cardioprotective effect of bosentan, another nonselective ET receptor antagonist, on the basis of decreased tissue MDA levels and increased intracellular creatine kinase-MB isoenzyme activity in the heart. Taken together, we may propose that tezosentan has protective and cell saving effects on myocardial injury subjected to aortic IR injury.

Tezosentan administration in the aortic IR + tezosentan group resulted in a further increase in ET-1 compared to that in the aortic IR. The increase in systemic ET-1 plasma levels in the aortic IR + tezosentan group is a sign of an effective receptor blockade by tezosentan in this set-
ting. This is supported by the findings of other studies using ET receptor antagonists (Herbert et al. 2001; Rossi et al. 2004; Uhllmann et al. 2005). This finding is also consistent with a considerable degree of ET_{B} receptor antagonism reducing the ET_{B} receptor-mediated clearance of circulating ET-1 (Rossi et al. 2004; Uhllmann et al. 2005).

Histological examination of heart specimens in the aortic IR + tezosentan group demonstrated that tezosentan ameliorated the deleterious effect of aortic IR. As shown in Table 2, the aortic IR + tezosentan group had less cardiac damage compared to the aortic IR group. This cardioprotective effect of tezosentan correlated with reduced tissue MDA and antioxidant enzymes, serum inflammatory markers, and TnI levels. Consisted with our results, Singh et al. (2006) observed significant improvements in histopathological changes in the myocardium by bosentan following myocardial IR in rats. They concluded that mixed ET-A and ET-B receptor antagonists hold a significant clinical potential for salvaging myocardial cells following IR injury.

**CONCLUSION**

From the results of this study using tezosentan, a mixed ET-receptor antagonist, we conclude that tezosentan reduces myocardial injury induced by aortic IR. During abdominal aortic IR injury-induced distant organ damage, tezosentan can ameliorate production of the ROS, activation of inflammatory response, and decrease myocardial injury. ET-receptor antagonists could be pharmacological tools for modifying the adverse effects on the myocardium during abdominal aortic surgery.

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**References**


