

Bosentan Reduces Myocardial Ischemia-Reperfusion Injury in Rats

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Abstract

Objectives: This study aimed to investigate the cardioprotective effects of bosentan, an endothelin receptor antagonist, against myocardial ischemia-reperfusion injury (MIRI) in rats.

Materials and Methods: Twenty-four adult Wistar-Albino rats were randomly divided into four groups: control, bosentan only, myocardial ischemia-reperfusion (MIR), and MIR-bosentan (MIR-B). Ischemia was induced by ligation of the left anterior descending coronary artery for 30 minutes, followed by 90 minutes of reperfusion. Bosentan was administered intraperitoneally at 30 mg/kg during ischemia in the MIR-B group. Histopathological evaluation assessed neutrophil infiltration, cardiomyocyte damage, tissue edema, and hemorrhage, while biochemical analyses measured total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), and paraoxonase-1 (PON-1) activity in myocardial tissue.

Results: The MIR group showed significantly increased histopathological injury scores, including neutrophil infiltration, cardiomyocyte damage, edema, and hemorrhage, compared to control and bosentan-only groups ($p<0.001$). Bosentan treatment significantly reduced these injury scores in the MIR-B group compared to the MIR group ($p<0.05$). Biochemically, the MIR group exhibited elevated TOS and OSI levels and reduced TAS and PON-1 activity, indicating oxidative stress.



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Bosentan administration significantly improved these parameters by lowering TOS and OSI levels, and by increasing TAS and PON-1 activity compared to the MIR group ($p < 0.05$).

Conclusion: In conclusion, bosentan demonstrated significant protective effects against MIRI by attenuating histological damage and oxidative stress in rat myocardium. These findings suggest that endothelin receptor antagonism with bosentan may offer a promising therapeutic approach to reduce myocardial injury following ischemia-reperfusion events such as those occurring during coronary artery bypass grafting. Further studies are needed to explore its clinical potential.

Keywords: Cardiovascular surgery, coronary arteries, heart

Introduction

Cardiovascular diseases (CVD) are the leading cause of death worldwide, with coronary heart disease (CHD) being the major contributor⁽¹⁾. In the 18th century, William Heberden first described angina pectoris in medical history, and later, CHD was identified as a condition caused by reduced blood flow in the coronary arteries⁽²⁾. By the late 19th century, it was established that coronary artery occlusion is fatal, and coronary thrombosis was linked to myocardial infarction (MI)⁽²⁾. CHD refers to a type of CVD resulting from atherosclerosis or atherosclerotic occlusion of the coronary arteries of the heart⁽²⁾. This condition leads to reduced blood flow in the coronary arteries, disrupting the balance between myocardial oxygen demand and supply⁽²⁾. Consequently, clinical symptoms of CHD occur such as a pressure-like sensation in the chest that radiates to the jaw and left arm⁽²⁾. This clinical presentation reflects acute myocardial infarction (AMI) and may progress to heart failure or death⁽³⁾. The two main goals of treatment are to medically relieve angina symptoms and to restore blood flow invasively⁽³⁾. There are two invasive methods to reestablish coronary blood flow: percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG)⁽³⁾. Although CABG provides collateral circulation in addition to revascularization and thus offers superior protection compared to PCI⁽³⁾, both revascularization techniques are widely available, particularly in Western countries. Rapid revascularization is essential to prevent permanent tissue damage⁽⁴⁾. However, reperfusion itself initiates ischemia-

reperfusion (IR) injury not only in the myocardium but also in distant organs⁽⁴⁾. Ischemia causes an increase in intracellular sodium, hydrogen, and calcium ions, resulting in cellular acidosis⁽⁵⁾. This leads to myocardial hypercontractility, depletion of adenosine triphosphate (ATP), mitochondrial damage, and myocardial stunning⁽⁵⁾. The generation of reactive oxygen species (ROS) begins with the activation of proapoptotic pathways⁽⁶⁾. Restoration of blood flow further contributes to ROS production and complement activation⁽⁶⁾. Therefore, assessing myocardial damage both histologically and biochemically is crucial. Measuring total oxidant status (TOS), total antioxidant status (TAS), and paraoxonase (PON) activity is useful for biochemical evaluation of damage^(7,8). Histological assessment, of tissue edema and hemorrhage is also valuable in evaluating tissue injury^(7,8).

Bosentan is the first endothelin (ET) receptor antagonist that has potential clinical applications⁽⁹⁾. Although bosentan is officially approved only for pulmonary arterial hypertension, the ET system is activated in many cardiovascular conditions such as congestive heart failure (CHF), essential hypertension, acute AMI, and atherosclerosis⁽⁹⁾. ET-1 is the most potent endogenous vasoconstrictor in the human body, being approximately 100 times more powerful than noradrenaline and 10 times more potent than angiotensin II⁽⁹⁾. ET-1 also exhibits pro-fibrotic, pro-inflammatory, and mitogenic activities in vascular tissues and the myocardium⁽⁹⁾. This underlies the potential beneficial effects of bosentan in myocardial ischemia-reperfusion injury (MIRI).

We aim to investigate the protective effects of bosentan against MIRI in rats. MIRI is a common complication following CABG in cardiac surgery. Bosentan may exert beneficial effects against MIRI. Conducting an animal study in this context may help clarify future treatment strategies to reduce MIRI.

Materials and Methods

Animals

This experimental study was conducted at the Gazi University Laboratory Animal Breeding and Experimental Research Center (GÜDAM) in accordance with ARRIVE guidelines. This study was approved by the Gazi University Local Ethics Committee for Animal Experiments (approval no: G.Ü.ET-22.062, date: 22.11.2023). All animals were housed and cared for according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Establishment of the Experimental Model

Anesthesia was induced by intramuscular injection of ketamine hydrochloride (50 mg/kg; Ketalar® vial, Parke-Davis, USA). The rats were placed in the supine position under a heat lamp. After shaving the surgical areas, a midline vertical cervical incision of approximately 1 cm was made. The trachea was exposed by blunt dissection, and a tracheostomy was performed using a 16G intra-catheter (Medipro Nova Cath®, İstanbul, Türkiye). Subsequently, the rats were connected to a mechanical ventilator (Harvard Apparatus Rodent Model Ventilator, Inspira ASV, Holliston, USA) and ventilated with 100% oxygen at a tidal volume of 10-15 mL/kg and a respiratory rate of 65-80 breaths per minute throughout the procedure. A left thoracotomy was performed through the fourth intercostal space. The pericardial sac was opened, and myocardial ischemia was induced by occlusion of the left anterior descending (LAD) coronary artery using an 8-0 Prolene suture.

In this study, 24 adult Wistar-Albino rats with an average weight of 250 g were used. The animals were randomly divided into four groups (n=6 per group):

Control group (C, n=6): Tracheostomy and thoracotomy were performed, but no myocardial IR was induced. The rats were maintained under anesthesia for 120 minutes and then sacrificed. Myocardial tissue samples were collected for histopathological and biochemical analyses.

Bosentan-only group (B, n=6): Following tracheostomy and thoracotomy, 30 mg/kg bosentan was administered intraperitoneally at the beginning of the procedure. No ischemia was induced. After 120 minutes under anesthesia, the rats were sacrificed, and myocardial tissue was collected.

IR group (MIR, n=6): After tracheostomy and thoracotomy, myocardial ischemia was induced by LAD occlusion for 30 minutes, followed by 90 minutes of reperfusion. The total experimental duration was 120 minutes. At the end of reperfusion, the rats were sacrificed and myocardial samples were collected.

Bosentan-treated IR group (MIR-B, n=6): Following tracheostomy and thoracotomy, 30 mg/kg bosentan was administered intraperitoneally prior to the induction of ischemia. After drug administration, LAD occlusion was applied for 30 minutes, followed by 90 minutes of reperfusion. At the end of the 120-minute experimental period, the rats were sacrificed under anesthesia, and myocardial tissue samples were harvested for further analysis.

Biochemical Assessment of Myocardial Tissue Samples

Following the completion of the reperfusion period, all animals were sacrificed under anesthesia and heart samples were harvested. To assess the TOS, TAS, and PON-1, half of the heart between the base and a plane at the mid-LAD level, parallel to the atrioventricular (AV) valve plane, was dissected. Parts of hearts were put in liquid nitrogen, then transferred to -80 °C, and stored until the day of assessment. TOS and TAS levels were measured spectrophotometrically with commercially available kits (Relassay, Türkiye)^(10,11), and the oxygenation saturation index (OSI) was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol

H₂O₂ equivalent/L)/TAS (μ mol Trolox equivalent/L)⁽¹²⁻¹⁴⁾. PON-1 activity was calculated from two consecutive spectrophotometrical measurements with a commercially available kit (Relassay, Türkiye).

Histopathological Assessment of Myocardial Tissue Samples

The part of the heart samples between the apex and mid-LAD level, which is parallel to the AV valve, is fixed in 10% buffered formalin for 48 hours. Fixation is followed by the routine tissue processing procedures to obtain paraffin tissue blocks. To that end, specimens were dehydrated through a graded series of alcohol, then were cleared in xylene. After being infiltrated with liquid paraffin, tissue samples were embedded in paraffin. From each heart, paraffin blocks, four 4 μ m-thick heart sections with 50 μ m intervals, were cut parallel to the base of the heart using a microtome (HistoCore MULTICUT, Leica, Germany). All sections were stained with hematoxylin and eosin (H&E) to evaluate the histopathological changes.

H&E-stained heart specimens were assessed under 200 $\times$ and 400 $\times$ magnifications using a light microscope (Leica DM 4000 B, Germany), and micrographs were taken using the software Leica LAS V4.12. Myocardial injury resulting from IR was assessed in terms of histopathological changes involving neutrophil infiltration, cardiomyocyte injury, interstitial edema, and hemorrhage. Scores ranging between 0 and 3 were assigned to reveal the severity of the individual histopathological changes, of which 0 indicating no change, 1 indicating weak changes, 2 indicating moderate changes, and 3 indicating severe changes. Then, scores of individual histopathological changes, and the total heart muscle injury score, ranging between 0 and 12, were compared between the groups as the sum of the other scores⁽¹⁵⁾.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) 22. The normal distribution of variables was examined visually (histograms and

probability plots) and analytically (Kolmogorov-Smirnov and Shapiro-Wilk tests). The results are presented as mean $\pm$ standard error. Data were evaluated using Kruskal-Wallis variance analysis. Significant variables were assessed using the Bonferroni-corrected Mann-Whitney U test. A Type I error level of 5% was set to indicate statistical significance with $p < 0.05$.

Results

Results of Biochemical Assessment

TAS and TOS levels, as well as OSI values, were found to be significantly different among the groups ($p < 0.001$ for all). TAS levels in the MIR group were significantly lower than in both group C and group B ($p < 0.001$ for both). TAS levels of the myocardial tissues in the MIR+B group were also significantly lower than in group C, and group B ($p = 0.007$ and $p = 0.020$, respectively); however, they were significantly higher compared to those in the MIR group ($p = 0.046$) (Table 1).

TOS levels in the MIR group were found to be significantly higher than those in groups C and B ($p < 0.001$, both). Similarly, TOS levels in the MIR+B group were notably elevated compared to those in group C and group B ($p = 0.023$ and $p = 0.008$, respectively), yet they were still significantly lower compared to those in the MIR group ($p = 0.003$) (Table 1).

Analysis revealed a significant increase in OSI values in the MIR group compared to groups C and B ($p < 0.001$, both). Although OSI values of the MIR+B group were considerably higher than those of group C and group B ($p = 0.005$ and $p = 0.009$, respectively), they were significantly lower than in the MIR group ($p < 0.001$) (Table 1).

PON-1 activity was also significantly different between the groups ($p = 0.003$). PON-1 activity in the MIR group was significantly lower, compared to group C and group B ($p = 0.001$, both), whereas the PON-1 activity in the MIR+B group was significantly greater than that in the MIR group ($p = 0.005$) (Table 1).

Table 1. Oxidative status parameters of cardiac tissue (mean $\pm$ SD)

	Group C (n=6)	Group B (n=6)	Group MIR (n=6)	Group MIR + B (n=6)	P**
TAS (mmol/L)	1.23 $\pm$ 0.15	1.19 $\pm$ 0.14	0.86 $\pm$ 0.10*, &	1.01 $\pm$ 0.09*, &, +	<0.001
TOS (μ mol/L)	8.18 $\pm$ 1.61	7.62 $\pm$ 2.38	14.95 $\pm$ 2.06*, &	11.04 $\pm$ 1.92*, &, +	<0.001
OSI	0.68 $\pm$ 0.20	0.65 $\pm$ 0.19	1.75 $\pm$ 0.32*, &	1.10 $\pm$ 0.27*, &, +	<0.001
PON-1 (U/L)	9.06 $\pm$ 2.57	9.19 $\pm$ 2.43	3.66 $\pm$ 1.69*, &	8.22 $\pm$ 3.10+	0.003

p**: Significance level with Kruskal-Wallis test; p<0.05. *p<0.05: Compared to group C; &p<0.05: Compared to group B; +p<0.05: Compared to group myocardial ischemia-reperfusion injury (MIR). Group C, control group; group B, bosentan group; group MIR, myocardial ischemia reperfusion group; group MIR+B, myocardial ischemia reperfusion and bosentan treatment group. TAS: Total antioxidant statu, TOS: Total oxidant status, OSI: Oxidative stres index, PON-1: Paraoxonase-1, SD: Standard deviation

Results of Histopathological Assessment

Histopathological evaluation of the heart specimens demonstrated a significant difference in neutrophil infiltration, cardiomyocyte injury, interstitial edema, and hemorrhage between the groups, collectively indicated by $p<0.001$ (Table 2). In the observation of H&E-stained heart muscle specimens from groups C and B, a slight interstitial edema and congestion were noted (Figure 1).

Examination of H&E-stained heart muscle samples revealed a much more pronounced neutrophil infiltration in the MIR group compared to groups C and B ($p<0.001$, both). Also, cardiomyocyte injury in the MIR group was greater than that in groups C and B ($p<0.001$, both). Interstitial edema was much more severe in the MIR group compared to groups C and B, with both comparisons showing $p<0.001$. Hemorrhage that was observed in the specimens of the MIR group was much more prominent than that observed in groups B and C ($p<0.001$, both) (Table 2, Figure 2).

The comparison of the scores for the histopathological changes in H&E-stained heart muscle specimens of groups revealed a significantly lower neutrophil infiltration in the MIR+B group compared to the MIR group ($p=0.003$). Additionally, cardiomyocyte injury in the MIR+B group was greater than that in group C and group B ($p=0.003$ and $p<0.001$, respectively); however, it was milder compared to the MIR group ($p=0.044$). Although interstitial edema in the MIR+B group was more severe than interstitial edema in groups C and B ($p=0.001$ and $p<0.001$, respectively), it was found to be significantly reduced compared to

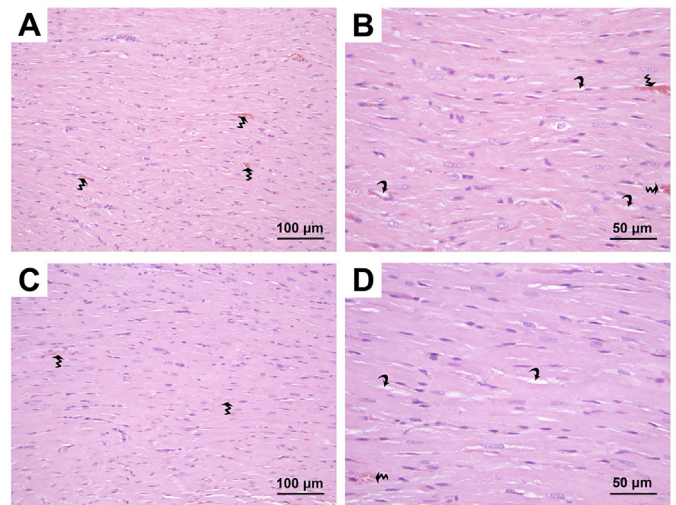


Figure 1. Micrographs of heart specimens from group C (A and B) and group B (C and D). Mild congestion (wavy arrows) and interstitial edema (curved arrows) are observed in sections of groups C and B. Stain: hematoxylin and eosin (H&E). Magnification is 200 $\times$ for images A and C; 400 $\times$ for images B and D.

interstitial edema in the MIR group ($p=0.029$). Also, hemorrhage in the MIR+B group was more apparent than that in group C and group B ($p=0.001$, both); however, it was less notable compared to that of the MIR group ($p=0.004$) (Table 2, Figure 3).

The total heart muscle injury score of the MIR group was found to be significantly higher than that of group C and group B ($p<0.001$, both). While the score of the MIR+B group was also significantly higher than those of group C and B ($p<0.001$, both), it was found to be significantly lower compared to that of the MIR group ($p<0.001$) (Table 2).

Table 2. Myocardial ischemia-reperfusion injury scores (mean $\pm$ SE)

	Group C (n=6)	Group B (n=6)	Group MIR (n=6)	Group MIR+B (n=6)	P**
Neutrophil infiltration	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	2.00 $\pm$ 0.44', &	0.67 $\pm$ 0.33+	<0.001
Cardiomyocyte injury	0.50 $\pm$ 0.22	0.33 $\pm$ 0.21	2.67 $\pm$ 0.33', &	1.83 $\pm$ 0.31', &, +	<0.001
Interstitial edema	0.67 $\pm$ 0.21	0.33 $\pm$ 0.21	2.83 $\pm$ 0.17', &	2.00 $\pm$ 0.37', &, +	<0.001
Hemorrhage	0.50 $\pm$ 0.22	0.50 $\pm$ 0.22	2.67 $\pm$ 0.21', &	1.67 $\pm$ 0.21', &, +	<0.001
Total heart muscle injury score	1.67 $\pm$ 0.61	1.16 $\pm$ 0.48	10.17 $\pm$ 0.98', &	6.17 $\pm$ 0.31', &, +	<0.001

p**: Significance level with Kruskal-Wallis test; p<0.05. 'p<0.05: Compared to group C; & p<0.05: Compared to group B; +p<0.05: Compared to group myocardial ischemia-reperfusion injury (MIR). Group C, control group; group B, bosentan group; group MIR, myocardial ischemia reperfusion group; group MIR+B, myocardial ischemia reperfusion and bosentan treatment group, SE: Standard error

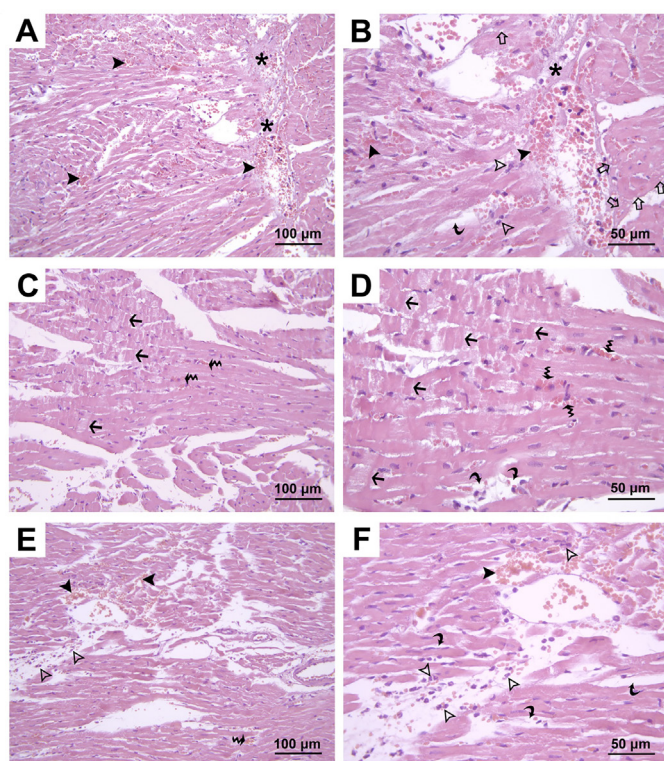


Figure 2. Micrographs of heart specimens from group MIR. Besides congestion (wavy arrows in images C, D and E), more pronounced interstitial edema (curved arrows in images B, D and F) than the other groups is noted. Varying degrees of cardiomyocyte injury are present in the myocardium so that the regions with no myocardial cellular integrity (asterisks in images A and B), myocardial cells with contraction bands (black arrows in images C and D), and myocardial cells exhibiting vacuoles (hollow arrows in image B) are visible. Hemorrhage (black arrowheads in images A, B, E, and F) and neutrophil infiltration (hollow arrowheads in images B, E, and F) are also notable. Stain: hematoxylin and eosin (H&E). Magnification is 200 $\times$ for images A, C and E; 400 $\times$ for images B, D and F. MIR: Myocardial ischemia-reperfusion injury

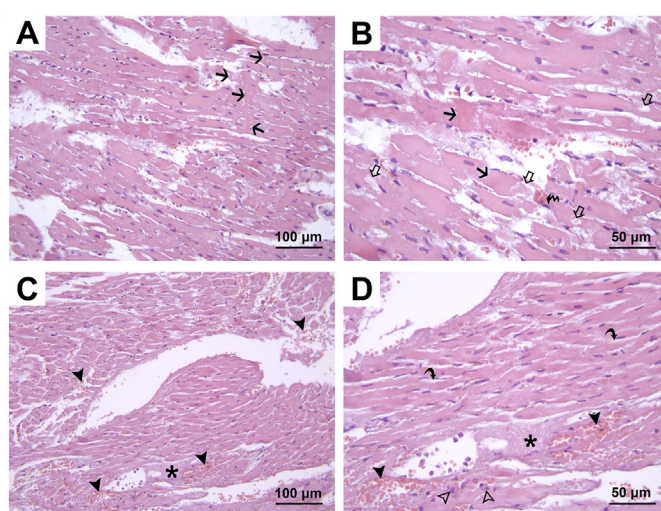


Figure 3. Micrographs of heart specimens from group MIR+B. Congestion (wavy arrow in image B), interstitial edema (curved arrows in image D), neutrophil infiltration (hollow arrowheads in image D), and hemorrhage (black arrowheads in images C and D) appear milder than the myocardial ischemia-reperfusion injury (MIR) group. Myocardial cells with contraction bands (black arrows in images A, B) and myocardial cells exhibiting cytoplasmic fragmentation, or vacuoles (hollow arrows in image B) are less widespread. Regions with no myocardial cellular integrity (asterisks in images C and D) are not as extensive as those seen in the MIR group. Stain: hematoxylin and eosin (H&E). Magnification is 200 $\times$ for images A and C; 400 $\times$ for images B and D

Discussion

During ischemia, lowered oxygen levels trigger ATP depletion, which activates anaerobic glycolysis, leading to increased hydrogen and lactate levels and resulting in acidosis⁽¹⁶⁾. Decreased ATP levels inhibit the sodium-potassium-ATPase enzyme, causing sodium and chloride accumulation and subsequent cell swelling⁽¹⁶⁾. Although

calcium levels increase, the calcium sensitivity of myofibrillar proteins decreases, impairing contraction⁽¹⁶⁾. Myocyte necrosis exacerbates the inflammatory response⁽¹⁶⁾. Ischemic myocytes and activated leukocytes initiate ROS generation, causing peroxidative damage to membrane phospholipids⁽¹⁶⁾. This process results in increased membrane leakage⁽¹⁶⁾. Consequently, oncosis and apoptosis begin⁽¹⁶⁾. Upon reperfusion, mitochondrial oxidative phosphorylation recovers quickly, but contraction returns gradually due to myocardial stunning⁽¹⁷⁾. During reperfusion, acidosis resolves rapidly through hydrogen efflux and sodium influx, followed by activation of the sodium-calcium exchanger; calcium accumulation then triggers cell death⁽¹⁷⁾. Subsequently, mitochondrial permeability transition pores open, triggering ROS generation and the release of proinflammatory factors such as tumor necrosis factor alpha, toll-like receptors, nuclear factor kappa B, and danger-associated molecular patterns⁽¹⁷⁾. These chemoattractant cytokines then recruit neutrophils into the reperfused area⁽¹⁷⁾. We aim to interrupt this vicious cycle, which leads to increased inflammation, elevated ROS production, and further cell damage.

The ET family consists of 21-amino-acid peptides and includes three types: ET-1, ET-2, and ET-3⁽⁹⁾. These ETs exert their effects by binding to ET_A and ET_B receptors⁽⁹⁾. ET_A receptors are located on smooth muscle cells and mediate the vasoconstriction effects of ET-1⁽⁹⁾. ET_B receptors are primarily found on endothelial cells, where they bind ET-1 and ET-3 and are responsible for vasodilation through the release of nitric oxide (NO) and prostacyclin^(9,18,19). The vasoconstriction effects of ET-1 occur in the pulmonary, coronary, renal, and systemic vascular beds⁽⁹⁾. Additionally, ET-1 acts as an agonist of the sympathetic nervous system and the renin-angiotensin-aldosterone system⁽⁹⁾. ET-1 also activates neutrophils, mast cells, and monocytes, which in turn stimulate a wide range of cytokines, explaining its role in inflammation⁽¹⁸⁾. Beyond its mitogenic, profibrotic, and proinflammatory activities in the myocardium and vascular beds, ET-1, negatively affects the heart through the hypertrophic effects mediated by noradrenaline and angiotensin II, as

well as the profibrotic effects of aldosterone⁽⁹⁾. Moreover, ET-1 levels are elevated in CHF and AMI^(7,20,21). ET-1 blood levels also correlate with one-year mortality after AMI^(9,19). Bosentan is a specific antagonist of ET-1 that blocks both ET_A and ET_B receptors⁽⁹⁾. However, bosentan is 100 times more selective for ET_A receptors, which accounts for its clear vasodilatory effects⁽⁹⁾. These properties of bosentan led us to consider its potential cardioprotective effects. Our study's histological and biochemical analyses demonstrated that bosentan significantly attenuates MIRI, thereby supporting its proposed cardioprotective role.

There are studies similar to ours in the literature. For instance, Gong et al.⁽²²⁾ found that bosentan has positive effects on neuronal ischemia and reperfusion. Similarly, Demirtaş et al.⁽²³⁾ demonstrated beneficial effects of bosentan on IR injury in the rat hind limb. Although their study focused on the hind limb⁽²³⁾ and ours concerns MIRI both studies reflect the role of ETs in ischemia-reperfusion injury. In this regard, Skovsted et al.⁽²⁴⁾ investigated the effect of ET-1 on MIRI in an experimental rat model. They found that the MEK-ERK-1 pathway plays an important role in MIRI through the transcription of ET-1⁽²⁴⁾. Ai et al.⁽²⁵⁾ reported different results on this topic, in their experimental rat model, they found that a combination of ET-1 and NO may reduce MIRI. In contrast to their findings, Tamareille et al.⁽²⁶⁾ showed that ET-1 release may be an early mediator of MIRI. They claimed that ET-1 release triggers calcium overload, which activates apoptosis, and therefore, blocking ET-1 could reduce MIRI⁽²⁶⁾. Their hypothesis aligns with our biochemical and histological findings. In our study, bosentan was administered prior to ischemia to block the early surge of ET-1 activity. Since ET-1 release during the onset of reperfusion has been shown to aggravate myocardial injury through vasoconstriction, calcium overload, oxidative stress, and inflammation^(24,26), the optimal clinical timing of bosentan administration in acute MI or CABG might be immediately before reperfusion (e.g., before coronary revascularization or aortic cross-clamp removal). Further studies are warranted to clarify this therapeutic window.

Additionally, several important studies have reported that bosentan has positive effects against MIRI, which supports our results. Wang et al.⁽²⁷⁾ demonstrated in an experimental study conducted on pigs that bosentan significantly reduces MIRI. They claimed that bosentan exerts cardioprotective effects and improves blood flow, especially in the reperfused area⁽²⁷⁾. We used a rat model in this study due to its cost-effectiveness and wide acceptance in preclinical IR research. Despite lower overall costs, our findings were consistent with those reported in more complex or higher-cost experimental models. In a different study, Wang et al.⁽²⁸⁾ focused on bosentan's cardioprotective effects, this time in rats, and reported similar findings. Bosentan showed cardioprotective effects on isolated rat hearts through ET inhibition. Consistent with our findings, Li et al.⁽²⁹⁾ reported that bosentan has positive effects on MIRI, protecting both the myocardium and endothelium. Gupta et al.⁽³⁰⁾ revealed that bosentan reduces oxidative stress and has beneficial effects against MIRI. Their study confirms the critical role of ET-1 during AMI and demonstrates that blocking its effects with bosentan confers cardioprotection⁽³⁰⁾. These findings are consistent with and supportive of the results of our study.

On the other hand, our study focused solely on the effects of bosentan, therefore, dose-dependent studies on this topic are needed. Additionally, potential side effects of bosentan, such as hepatotoxicity, should be considered⁽³¹⁾.

In conclusion, this study demonstrates that bosentan, an ET receptor antagonist, exerts significant protective effects against MIRI in rats. Histopathological analysis revealed that bosentan administration during IR notably reduced neutrophil infiltration, cardiomyocyte damage, tissue edema, and hemorrhage compared to untreated IR groups. Biochemically, bosentan treatment improved the oxidative balance by decreasing TOS and OSI while increasing TAS, indicating a reduction in oxidative damage. These findings support the role of ET-1 in exacerbating IR injury through vasoconstriction, inflammation, and oxidative stress, and highlight bosentan's potential as a cardioprotective agent

by antagonizing these effects. Given the clinical relevance of IR in CABG and other cardiac interventions, bosentan may represent a promising therapeutic strategy to mitigate myocardial damage and improve outcomes. Further studies are warranted to explore its clinical applicability and long-term benefits in human subjects.

Study Limitations

This study provides valuable insights into the cardioprotective effects of bosentan in a rat model of MIRI. However, several limitations must be acknowledged. First, the sample size was relatively small, with six animals per group, which may limit the statistical power and generalizability of the results. Although statistically significant differences were observed, a larger sample size would provide more robust and reproducible outcomes.

Second, the study was conducted exclusively on healthy, young adult male Wistar-Albino rats. The exclusion of female subjects and animals with comorbid conditions (e.g., diabetes, hypertension, or hyperlipidemia) may not fully reflect the complex pathophysiology of IR injury observed in human patients, particularly those undergoing CABG. Future studies should incorporate models with relevant comorbidities to enhance translational value.

Third, bosentan was administered as a single intraperitoneal dose, and no dose-response relationship was explored. The absence of multiple dosing regimens and pharmacokinetic analyses limits the ability to determine the optimal therapeutic window and systemic effects of bosentan. Moreover, the study did not evaluate potential adverse effects such as hepatotoxicity, which has been reported in clinical settings⁽³¹⁾. In clinical practice, bosentan use has been associated with liver enzyme elevations, peripheral edema, anemia, headache, and hypotension. Preventive strategies include careful patient selection, baseline and periodic monitoring of liver function tests, avoidance of concomitant hepatotoxic medications, and timely dose adjustment or discontinuation in case of significant aminotransferase elevation. Although these adverse effects were not observed in our short-term

experimental protocol due to single-dose administration and limited observation time, they represent an important consideration for any future translational or clinical applications.

Another limitation is that we did not directly measure myocardial or plasma ET-1 levels. Instead, we focused on oxidative stress parameters (TAS, TOS, OSI, and PON-1) and histopathological injury scores to evaluate the extent of myocardial damage and the protective effect of bosentan. The omission of ET-1 measurement was primarily due to technical and financial limitations, as specific assays for ET-1 were not available during the study period. Nevertheless, direct measurement of ET-1 would provide valuable mechanistic insight, and future studies incorporating ET-1 quantification are warranted to better elucidate the molecular pathways underlying bosentan's cardioprotective effects.

Fourth, the observation period after reperfusion was limited to 90 minutes. Myocardial damage due to reperfusion injury may evolve over a longer period, and the short-term follow-up may not capture delayed histological or biochemical changes. Long-term assessments, including survival, cardiac function, and fibrotic remodeling, would provide a more comprehensive evaluation of bosentan's cardioprotective effects.

Lastly, although biochemical and histopathological endpoints were thoroughly assessed, molecular mechanisms underlying bosentan's protective effects such as its influence on ET-1 receptor signaling, inflammatory cytokine expression, or mitochondrial pathways were not investigated. Future investigations incorporating molecular analyses may clarify the specific cellular pathways through which bosentan exerts its effects during IR injury.

Despite these limitations, the findings of this study lay a foundation for further research and support the potential utility of e ET receptor antagonism as a cardioprotective strategy.

Conclusion

In this experimental study, bosentan a dual ET receptor antagonist demonstrated significant cardioprotective effects against MIRI in rats. The administration of bosentan during the ischemic phase led to a marked attenuation in histopathological scores of tissue injury, including neutrophil infiltration, cardiomyocyte necrosis, interstitial edema, and hemorrhage. In parallel, bosentan significantly modulated oxidative stress by lowering TOS and OSI, while increasing TAS and PON-1 activity.

These findings suggest that antagonism of ET-1 receptors can effectively disrupt the pathophysiological cascade triggered by ischemia and reperfusion, characterized by vasoconstriction, inflammation, and oxidative damage. Given the central role of ET-1 in cardiovascular pathology, bosentan may represent a promising pharmacological intervention to limit myocardial injury in settings such as CABG or acute MI.

The translational relevance of these findings may pave the way for novel cardioprotective strategies in the perioperative management of myocardial revascularization procedures.

While our results provide compelling preclinical evidence supporting the efficacy of bosentan in ameliorating MIRI, further research including dose-response studies, long-term functional assessments, and clinical trials is necessary to validate its therapeutic potential and safety profile in human subjects.

Ethics

Ethics Committee Approval: This study was approved by the Gazi University Local Ethics Committee for Animal Experiments (approval no: G.Ü.ET-22.062, date: 22.11.2023). All experimental procedures were conducted at a certified animal research laboratory, Gazi University Center for Experimental Research and Practice (GÜDAM), in Ankara, Türkiye, in compliance with national and institutional ethical guidelines.

Informed Consent: All animals were housed and cared for according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Demirtaş H, Özer A, Yiğman Z, Concept: Yiğman Z, Demirtaş H, Gülcan MB, Özer A, Dursun AD, Küçük A, Arslan M, Design: Yiğman Z, Demirtaş H, Gülcan MB, Özer A, Dursun AD, Küçük A, Arslan M, Data Collection and/or Processing: Yiğman Z, Demirtaş H, Gülcan MB, Özer A, Dursun AD, Küçük A, Arslan M, Analysis and/or Interpretation: Yiğman Z, Demirtaş H, Gülcan MB, Özer A, Dursun AD, Küçük A, Arslan M, Literature Search: Demirtaş H, Arslan M, Writing: Demirtaş H, Arslan M, Gülcan MB, Yiğman Z.

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