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Original Article

The effect of Edaravone on lung injury after lower limb ischemia-reperfusion in a rat model: Biochemical and histopathological insights

Mehmet Ceber[1], Ilker Akar[1], Ilker Ince[2], Cemal Aslan[1], Sameh Alagha[3]

[1]Tokat Gaziosmanpaşa University School of Medicine, Cardiovascular Surgery Department, Tokat, Türkiye
[2]Ankara Etlik City Hospital, Cardiovascular Surgery Department, Ankara, Türkiye
[3]Bozok University Hospital School of Medicine, Cardiovascular Surgery Department, Yozgat, Türkiye

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Abstract

Aim: Lower limb ischemia/reperfusion leads to distant organ dysfunction, notably affecting the lungs, resulting in respiratory failure and significant morbidity and mortality. Edaravone is a new free radical scavenger and has attracted the attention of researchers. This study aims to examine edaravone's influence on lung injury arising from lower limb ischemia-reperfusion.
Material and Methods: Forty male Wistar rats were categorized into groups: Sham, Ischemia/Reperfusion (IR), Solvent, and Edaravone. The infrarenal abdominal aorta was clamped for 120 minutes and then reperfused for another 120 minutes. Edaravone was administered 30 minutes before the ischemic event. Then we analyzed serum and lung tissue samples for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and nitric oxide (NO) levels. Furthermore, a thorough histopathological assessment was conducted.
Results: In the IR group, edaravone notably reduced serum and lung MDA levels. While significant differences in serum SOD and NO levels existed between the IR and edaravone groups, similar differences were not found in the lung tissue samples. There appeared to be no significant impact of edaravone on serum and lung GPx levels. The histopathological investigation revealed that the lung injury score was significantly higher in the IR group compared to the control group, a difference mitigated by edaravone treatment.
Conclusion: Our findings suggest that edaravone mitigates lower limb ischemia-reperfusion-induced lung injury, both biochemically and histopathologically. Our findings imply the potential utility of this agent in the context of acute ischemia-reperfusion injury following vascular surgery.

Keywords: Lower extremity, edaravone, lung injury, ischemia, reperfusion

INTRODUCTION

Acute ischemia-reperfusion injury within the lower extremities frequently arises in situations like aortic surgery, where temporary cross-clamping of the abdominal aorta or acute occlusions of the femoral artery is encountered. Extensive research has demonstrated significant pathological alterations, including in the lungs, following the reperfusion of lower extremity ischemia due to aorta cross-clamping. In certain instances, patients may require prolonged ventilator and inotropic support [1]. The interruption of blood perfusion during ischemia leads to cellular energy depletion, and toxic metabolite buildup, and triggers biochemical reactions such as cell dysfunction, cell demise, and tissue necrosis [2-5]. Reperfusion, signifying the restoration of blood flow to ischemic tissue, not only incites local tissue

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Corresponding Author: Sameh Alagha, Bozok University Hospital School of Medicine, Cardiovascular Surgery Department, Yozgat, Türkiye
Email: samehalagha@gmail.com

damage but also contributes to distant organ injury by introducing accumulated free oxygen radicals and toxic metabolites into the systemic circulation [6,7]. Among these distant organ injuries, the lungs are particularly susceptible following lower extremity ischemia-reperfusion (IR) [8]. Activated neutrophils aggregate in lung tissue after being stimulated by pro-inflammatory cytokines released by extremity muscles during ischemia, considerably contributing to lung damage [9].

Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one, C10H10N2O, MCI-186) emerges as a potent novel scavenger of free oxygen radicals. This drug inhibits phospholipase A2 activation and platelet-activating factor (PAF) production, reducing pulmonary edema and leukocyte extravasation [10]. Previous studies have found that administration of edaravone prior to reperfusion reduces reperfusion injuries and cardiac damage in ischemia-reperfusion animal models. Because of these properties, edaravone has been identified as an effective, morphological and cytological protective agent [11,12]. In addition, a recently published study has investigated the renoprotective effects of edaravone in a IR rat model [13]. However, its effects on lung damage following reperfusion of ischemic limb remain unknown. In this study, we aim to investigate the potential of edaravone in preventing lung damage caused by ischemia-reperfusion in a lower limb IR model.

MATERIAL AND METHODS

Animals and Study Groups

We conducted an experimental study using a randomized controlled design, which involved four distinct groups. Our investigation centered on examining the levels of Serum and Lung Tissue SOD (Superoxide Dismutase) as a potential precursor. We enlisted a total of 40 rats, with each group consisting of 10 rats, while maintaining a study power of 80%, a 5% margin of error, and an effect size of 0.58. To allocate the rats into these groups, we employed a straightforward randomization process. No blinding method was employed. Rats that met the study conditions were selected using simple random sampling method. A total of 40 Wistar rats, each weighing between 250-350 grams, were enlisted for this investigation. Employing a process of simple randomization, the rats were allocated into four distinct groups.

Group 1 (sham group, n=10): In this group, laparotomy was conducted without any occlusion of the aorta.

Group 2 (ischemia/reperfusion–IR group, n=10): For this group, a clamp was applied to the infrarenal abdominal aorta for 120 minutes. Following this, the clamp was removed, and a 120-minute period of reperfusion ensued.

Group 3 (solvent group, n=10): This group received intravenous solvent at the same time point as the administration of edaravone in Group 4.

Group 4 (edaravone group, n=10): Rats in this group were administered intravenous (IV) edaravone at a dosage of 6mg/kg (Sigma-Aldrich, St. Louis, MO, USA), thirty minutes before the initiation of ischemia. Subsequently, the same surgical procedure as executed in the I/R group was followed.

Drug Preparation

Edaravone was solubilized in 1 N NaOH and adjusted to a pH range of 7.34 to 7.35 through titration with 1 N HCl, rendering it suitable for parenteral administration.

Surgical Procedure and Technique

The experiment was performed on rats according to the guidelines provided by the experimental animal laboratory. All surgical procedures were carried out with the animals under the influence of ketamine (50mg/kg) and xylazine (5mg/kg) anesthesia, administered intraperitoneally. No endotracheal intubation or mechanical ventilation was employed. Access to the infrarenal abdominal aorta was achieved through a midline laparotomy using a transperitoneal approach. To ensure anticoagulation, 400 U/kg of heparin was administered. Throughout the experiment, 10ml/kg of saline (NaCl) was infused through the tail vein. A microvascular clamp designed to minimize trauma was carefully placed on the infrarenal abdominal aorta. Subsequent to the clamping, an injection of 5 ml of saline was introduced into the intraperitoneal space. The laparotomy was subsequently sutured shut using 3/0 silk sutures.

After 120 minutes of ischemia, a reperfusion period of 120 minutes was employed [14]. The occurrence of aortic ischemia was ascertained by the absence of pulsation upon clamping, while successful reperfusion was indicated by the restoration of aortic pulsation post-clamp removal. At the end of the experiment, a median sternotomy was conducted across all groups. Blood samples were collected through intracardiac puncture and placed in biochemistry gel tubes. Both lungs were then excised. Subsequently, the rats were euthanized.

Blood samples underwent centrifugation, and the resulting serum samples were preserved at -80°C for subsequent biochemical analysis. One lung was immersed in a 10% formaldehyde solution for histopathological examination, while the other lung tissue was stored at -80°C for future biochemical analysis. In both tissue and serum samples, levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and nitric oxide (NO) were quantified. Additionally, a comprehensive histopathological assessment was conducted.

Biochemical Evaluation of Serum Samples

The activities of the SOD and GPx enzymes, as well as the levels of MDA and NO, were determined using specific assay kits. The Superoxide Dismutase Assay Kit (No. 706002; Cayman Chemical, Ann Arbor, MI, USA) was utilized for SOD activity measurement, while the Glutathione Peroxidase Assay Kit (No. 703102; Cayman Chemical, Ann Arbor, MI, USA) was employed for GPx enzyme activity assessment. The levels of MDA were

quantified using the TBARS Assay Kit (No. 10009055; Cayman Chemical, Ann Arbor, MI, USA), and NO levels were measured using the Nitrate/Nitrite Colorimetric Assay Kit (No. 780001; Cayman Chemical, Ann Arbor, MI, USA). In each case, the colorimetric method recommended by the manufacturer's instructions was followed.

Biochemical Evaluation of Lung Tissue Samples

Following the determination of the weight of each lung tissue, homogenization was carried out using 50 mM pH 7.4 phosphate buffer. An Ultra Turrax IKA brand T 18 homogenizer was employed for a 2-minute homogenization process. Subsequently, the obtained materials underwent measurement of SOD, GPx, MDA, and NO levels, utilizing the same assay kits utilized for serum specimens.

Histopathological Examination

Tissue samples procured from all study groups underwent standard follow-up procedures after being fixed in 10% formaldehyde. These tissues were subsequently embedded in paraffin blocks, yielding sections with a thickness of 4µm. The sections were then subjected to deparaffinization and stained using Hematoxylin-Eosin, facilitating their examination under a light microscope.

For the assessment of the severity of lung injury through pathological analysis, a modified iteration of the scoring system developed by Uysal and colleagues was applied. According to this system, various parameters, including neutrophil concentration, alveolar congestion, and interstitial edema within the lung tissue, were evaluated semi-quantitatively. The scoring scale employed was as follows: 0 indicated no change; 1 indicated mild focal changes; 2 suggested moderate multifocal changes; 3 denoted prominent focal changes; and 4 represented diffuse and significant changes.

Ethical Statement

All procedures were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986A. These protocols were authorized by the Committee on the Ethics of Animal Experiments at our university hospital, with the designated permit number 51879863-06, issued on 08.01.2015.

Statistical Analysis Method

Descriptive statistics, including mean and standard deviation, were applied to summarize the continuous variables. The normality of quantitative variables was examined with Shaphiro Wilk's test. Given that the number of independent groups exceeded two, the "One-Way ANOVA test" was employed. In instances where the ANOVA test yielded significance, subsequent paired comparisons were conducted using the Tukey HSD post-hoc test. Statistical significance was established at a threshold of $p<0.05$. These analyses were carried out using statistical software package (IBM SPSS Statistics 19, SPSS Inc., an IBM Co., Somers, NY).

RESULTS

Biochemical Results

Serum and Lung Tissue MDA Levels

In contrast to the control group, both the IR group and the solvent group exhibited elevated serum MDA levels, whereas the edaravone group demonstrated lower levels. Upon closer examination through post-hoc analysis, a statistically significant reduction in serum MDA levels was observed within the edaravone group (M=0.63±0.39, P=0.002). These results are summarized in Table 1 and depicted in Figure 1a.

Regarding MDA levels in lung tissue samples, a marked decrease was noted in the edaravone group when compared to the IR group (M=1.24±0.24 vs. 1.89±0.49, respectively, $p<0.001$). This data is presented in Table 1 and illustrated in Figure 1b.

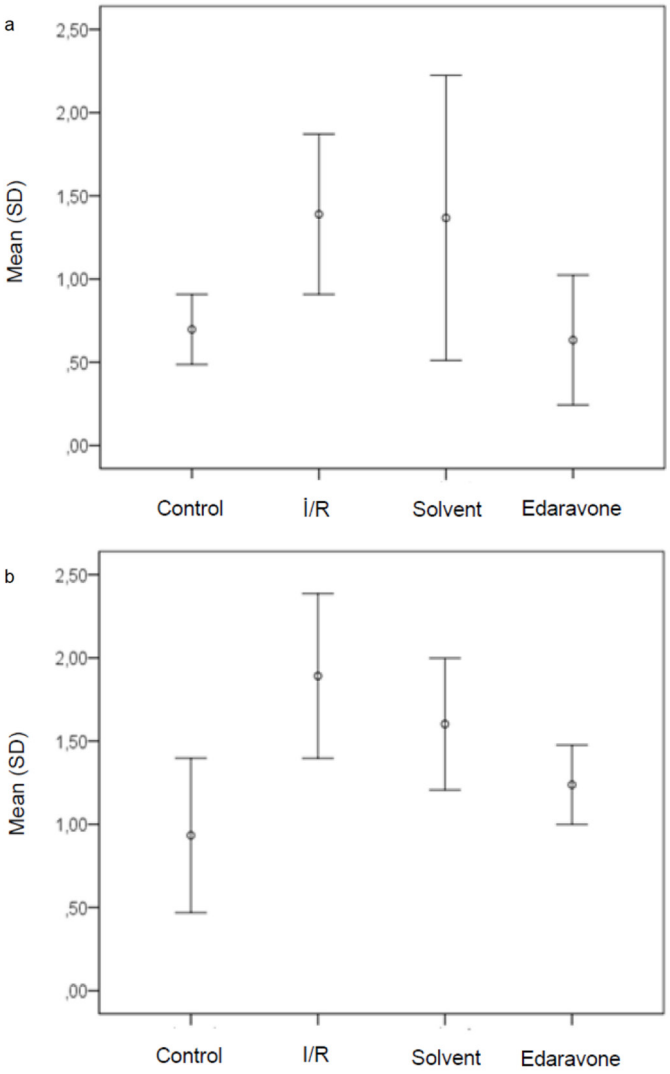

Figure 1. Comparison between groups according to MDA levels; **1a.** serum MDA levels; **1b.** lung tissue MDA levels

Serum and Lung Tissue SOD Levels

In comparison to the control group, both the IR group and the solvent group displayed reduced serum SOD levels. However, this variation between the groups did not yield statistical significance. Notably, the Edaravone group exhibited significantly higher serum SOD values compared to both the IR and solvent groups (M=4.77±0.80, P=0.020). These findings are summarized in Table 1 and depicted in Figure 2.

Likewise, when considering lung SOD levels, a pattern emerged where the IR and solvent groups showed lower levels compared to the control group, while the edaravone group demonstrated elevated levels. However, these distinctions between the groups did not reach statistical significance (P=0.897).

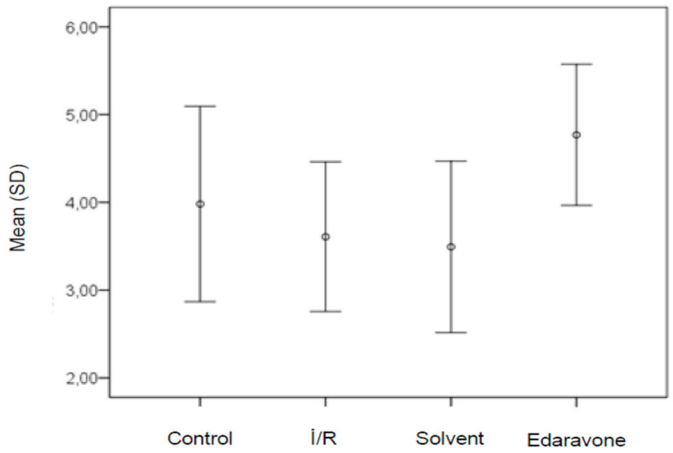

Figure 2. Comparison between groups according to serum levels of SOD

Serum and Lung Tissue Gpx Levels

Compared to the control group, both the IR group and the solvent group exhibited decreased serum GPx levels. Conversely, the edaravone group displayed higher GPx levels when compared to the IR and solvent groups. However, these variations between the groups did not reach statistical significance (P=0.097). Similarly, no statistically significant distinctions were observed among the groups with regard to GPX levels in lung tissue samples (P=0.762). These outcomes are outlined in Table 1.

Serum and Lung Tissue NO levels

Compared to the control group, both the IR group and the solvent group demonstrated elevated serum NO values. While the rise in the IR group did not achieve statistical significance, the increase observed in the solvent group was indeed statistically significant. Within the Edaravone group, serum NO levels were lower than those in both the IR and solvent groups. However, this difference within the edaravone group did not attain statistical significance when compared to the IR group. Nevertheless, a statistically significant distinction emerged when comparing the edaravone group to the solvent group (M=23.4±8.28 vs. 38.53±19.78, respectively, P=0.046). These results are presented in Table 1 and illustrated in Figure 3.

Turning attention to lung NO values, it became evident that the IR group and the solvent group exhibited higher levels in comparison to the control group. Conversely, lung NO levels were lower in the edaravone group compared to these two groups. Nonetheless, these differences among the groups did not reach statistical significance (P=0.874).

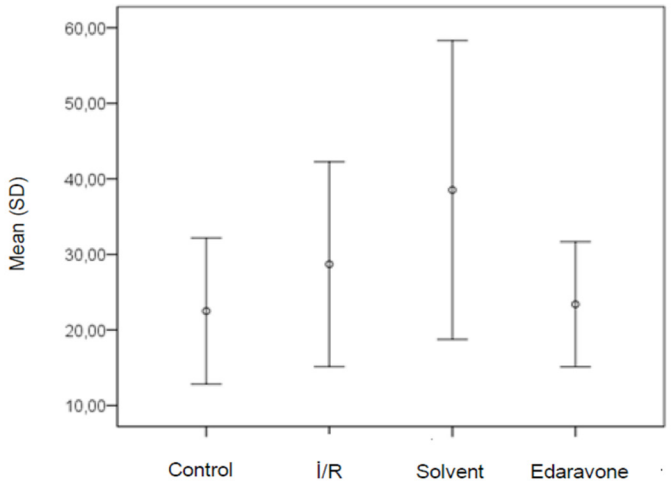

Figure 3. Comparison between groups according to serum levels of NO

Table 1. Distributions of quantitative variables according to groups

	Mean (SD)				p-value
	Sham (n=10)	IR (n=10)	Solvent (n=10)	Edaravone (n=10)	
Injury score	1.30±0.48[a]	2.70±0.67[b]	2.40±0.7b[c]	1.90±0.57[ac]	**<0.001**
SOD (serum)	3.98±1.11[ab]	3.61±0.85[a]	3.49±0.98[a]	4.77±0.80[b]	**0.020**
GPx (serum)	9.1±3.09	8.28±4.76	8.48±5.08	13.96±8.36	0.097
NO (serum)	22.5±9.66[a]	28.71±13.56[ac]	38.53±19.78[bc]	23.4±8.28[a]	**0.046**
MDA (serum)	0.7±0.21[a]	1.39±0.48[b]	1.37±0.86[b]	0.63±0.39[a]	**0.002**
SOD (Tissue)	4.84±1.62	4.70±1.26	4.74±1.40	5.11±0.76	0.897
GPx (Tissue)	20.76±7.69	23.14±7.72	20.58±7.98	19.67±6.55	0.762
NO (Tissue)	44.79±19.20	48.31±18.28	45.39±20.20	41.50±15.49	0.874
MDA (Tissue)	0.93±0.46[a]	1.89±0.49[b]	1.6±0.40[b]	1.24±0.24[a]	**<0.001**

Data are shown as mean±standard deviation. For groups, different superscripts (a,b,c) in the same row (ANOVA) indicate a statistically significant difference. SOD: superoxide dismutase, GPx: glutathione peroxidase, MDA: malondialdehyde, NO: nitric oxide

Histopathological Findings

The histological examination of specimens from the sham group exhibited a normal structural appearance (Figure 4a), with an average lung injury score of 1.30±0.48. Conversely, the IR group displayed pronounced thickening of alveolar septa, widespread interstitial edema, lung tissue congestion, and an elevated neutrophil count (Figure 4b), contributing to an elevated mean lung injury score of 2.70±0.67 within this group.

In contrast to the ischemia-reperfusion group, the Edaravone group demonstrated a reduction in interstitial edema, congestion, and polymorphonuclear leukocyte (PNL) presence (Figure 4d). The mean lung injury score in this group was measured at 1.90±0.57.

On the other hand, the solvent group, when compared to the edaravone group, displayed an increase in interstitial edema, congestion, and PNL count (Figure 4c). The mean lung injury score for this group reached 2.40±0.7.

Importantly, the lung injury score of the edaravone group was markedly lower than both the IR group and the solvent group. This difference between the groups held statistical significance ($p<0.001$), as outlined in Table 1.

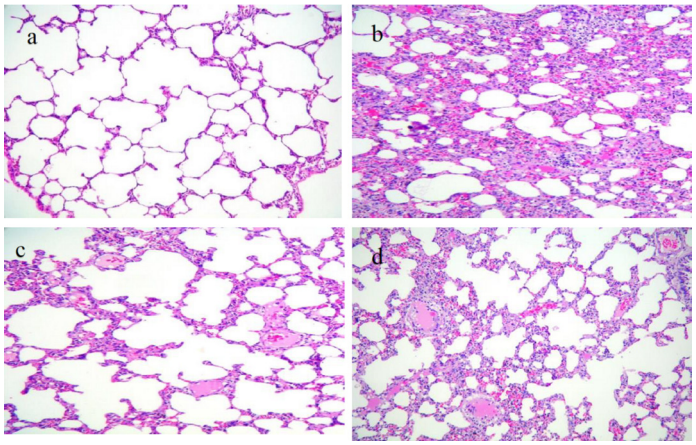

Figure 4. Lung tissue specimens in different groups; **4a.** Sham group (HE X 200); **4b.** Ischemia/reperfusion group (HE X 200); **4c.** Solvent group (HE X 200); **4d.** Edaravone group (HE X 200)

DISCUSSION

The distant organ injury observed after the ischemia-reperfusion in the lower extremities is still the nightmare of surgeons. Until today, several pharmacological agents were used to prevent the adverse effects of free oxygen radicals, which are mostly responsible for ischemia-reperfusion injury. Edaravone, which is produced synthetically, is a new and powerful free oxygen radical scavenger. In Japan and countries in East Asia, it is currently used in the treatment of acute cerebral infarcts and cerebrovascular events. Animal and human studies showed that edaravone scavenged free oxygen radicals after brain ischemia and inhibited the proinflammatory response. Studies also demonstrated that the brain edema and infarct, which developed as a result of the neuronal damage and endothelial cell death due to the inflammation after ischemia, could be improved with edaravone. In addition to these cerebral activities, it has also a preventive effect on oxidative damage in the extracerebral organs [10].

Ischemia-reperfusion injury spurs the generation of free radicals that wield direct influence on cellular membrane lipids, triggering lipid peroxidation due to their pronounced reactivity. Lipid peroxidation follows a chemical chain reaction instigated by Reactive Oxygen Species (ROS), involving the oxidation of polyunsaturated fatty acids (like arachidonic acid, linoleic acid, and linolenic acid) integral to cellular membrane composition. The hydroxyl radical, an especially noxious ROS, readily interacts with diverse cellular molecules including DNA, proteins, and carbohydrates. Interaction with cellular membrane lipids induces lipid peroxidation through H+ transfer from lipid molecules. This peroxidation sets off a chain reaction, persisting until chain completion or interruption by an antioxidant. The outcome of lipid peroxidation is the transformation of lipid hydroperoxides into aldehydes and other carbonyl compounds.

Research specifically targeting IRI has consistently indicated an elevation in MDA levels after IRI occurrence [15,16]. Nazlı et al. [17] conducted an experiment employing a rabbit model of spinal cord ischemia-reperfusion, revealing significantly reduced MDA levels in the cilostazol group compared to the IR group, wherein MDA levels had surged in contrast to the control. Huang et al. [18] investigated the effects of hydrogen-rich saline on IRI in the skeletal muscle tissue of rats and found that MDA levels in the serum and muscle tissue were higher in the IR group compared to the control group.

Another study investigated the impact of erythropoietin on ischemia-reperfusion injury (IRI) within rat lungs, revealing elevated MDA levels within lung tissue in the IR group [19]. In our study, we did not detect any difference between the IR and solvent groups. Nevertheless, MDA levels in serum and lung tissue were distinctly elevated in both the IR and solvent groups when contrasted with the sham group.

In an experimental study conducted by Zhou et al. [20], oxidative stress was induced in rat brains, revealing considerably elevated MDA levels within the brain tissue of the IR group when compared with the control group. Notably, in the same study, rats treated with edaravone exhibited significantly diminished MDA levels compared to the IR group. Meanwhile, a similar study involving isolated rat lungs, as conducted by Reyes et al. [21] observed significantly higher MDA levels within the lung tissue of the IR group and significantly reduced levels in the edaravone-administered group. Furthermore, Ito et al. [14] explored edaravone's protective efficacy in lung injury resulting

from an intestinal ischemia-reperfusion model involving 120 minutes of reperfusion post-superior mesenteric artery occlusion. This study employed an intravenous dose of 6mg/kg edaravone, mirroring our study's reference dose after reperfusion. Outcomes revealed significantly lowered MDA levels within lung tissue of the edaravone-treated group versus the IR group. Similarly, in our investigation, the IR and solvent groups displayed markedly elevated MDA levels in both serum and lung tissue, whereas the edaravone group exhibited substantial reductions. Edaravone achieves this effect by inhibiting lipid peroxidation, a pivotal facet of IRI [22,23].

Antioxidant enzymes are pivotal in combating free oxygen radicals, with SOD holding a significant role. SOD's effectiveness hinges on its catalytic function during the conversion of superoxide to hydrogen peroxide. Numerous studies involving IR models have consistently showcased diminished SOD levels in serum and tissue samples relative to control groups. Huang et al. [18] for instance, observed decreased SOD levels in both serum and muscle tissue of a rat skeletal muscle ischemia-reperfusion model's IR group, compared to controls. Notably, the hydrogen-rich saline group exhibited significantly elevated SOD levels in contrast to the IR group. Similar experimental investigations highlight analogous trends, with various studies reporting reduced SOD levels in serum and tissue samples across IRI models [14,17,20,24].

In our study, serum SOD values exhibited a notable reduction in both the IR and solvent groups, whereas the edaravone group showed a significant increase compared to the IR groups. As for lung tissue SOD levels, they were diminished in the IR group relative to the control group and elevated in the edaravone group versus the IR group. However, the disparities in lung tissue SOD levels didn't achieve statistical significance among the groups. Yurekli et al. [25] explored the impact of pheniramine on brain injury stemming from lower extremity ischemia-perfusion. Following a 24-hour reperfusion after a 1-hour ischemia, brain tissue SOD levels were notably lower than the control group. This observation might be attributed to the comparatively brief reperfusion duration of two hours in our study, contributing to the lower SOD levels observed in lung tissue.

GPx, another important antioxidant enzyme involved in the neutralization of free oxygen radicals, is dependent on its antioxidant ability to reduce H_2O_2 to H_2O. GPx levels consistently decreased in IR groups compared to control counterparts in various experimental studies employing IR models. In their research on the effects of pheniramine on the IRI in rat brain tissue, Yurekli et al. [25] found significantly lower GPx levels in the IR group compared to controls. Notably, the pheniramine group had significantly higher GPx levels. Significant decreases in GPx levels were reported in both blood and spinal cord tissue of the IR group, compared to controls, in another experimental investigation focusing on cilostazol's effects on spinal cord IRI. Surprisingly, the cilostazol group showed a considerable rise in GPx levels, emphasizing its antioxidant potential [17].

In their experimental IRI study, Zhou et al. [20] investigated the effect of edaravone on the brain tissue of rats, revealing substantially decreased GPx levels in the brain tissue of the IR group relative to controls. Notably, the edaravone-treated group showcased pronounced elevation in GPx levels when compared to the IR group. Within our study, GPx levels in both serum and lung tissue exhibited a reduction in both IR and solvent groups, while the edaravone group demonstrated elevated GPx levels compared to the IR groups. However, these distinctions failed to attain statistical significance. The aforementioned study by Yurekli and colleagues underscored the significant reduction of both GPx and SOD levels within the IR group. Additionally, in another study [17], the effects of cilostazol on ischemia-reperfusion injury in the spinal cord were investigated, and significantly low GPx levels were determined in tissue samples obtained after 72-hour reperfusion following a 30-minute ischemia in the IR group.

Nitric oxide is produced by NOS in the endothelial cells and has several beneficial effects, but if it is produced excessively and carries an unpaired electron, it acts like ROS and may have hazardous effects. The effects of NO in ischemia-reperfusion injury are not elucidated yet. Currently, there are studies showing its protective effects in IRI as well as other studies showing its hazardous effects [26].

Kirisci et al. [27] conducted a study investigating the effects of adrenomedullin on IRI in the skeletal muscles of rats. Their findings disclosed a substantial increase in NO levels within the muscle tissue of the IR group compared to controls, while the adrenomedullin-administered group exhibited marked NO level reduction in the IR group. Another experimental study [28] centered on melatonin's influence on IRI in rat lung tissue. This study elucidated a significant rise in NO levels within the lung tissue of the IR group when contrasted with controls. In contrast, the melatonin group demonstrated notable diminishment in NO levels.

In our study, serum NO levels increased significantly in the IR group while decreasing significantly in the edaravone group compared to the IR group. In terms of lung tissue, the IR group had higher NO levels, whereas the edaravone group had lower levels. However, the differences in tissue NO levels found among groups did not reach statistical significance.

Clinically, lung injury becomes evident as heightened vascular permeability within lung tissue, leading to acute respiratory failure through the development of lung edema [29]. There are several experimental studies in the literature focused on the pathological changes that emerged in the lung tissue as a result of the IRI in the lower extremities. Wu et al. [30], for instance,

investigated the effect of erythropoietin on lung injury related to the IRI in rats and determined a significant increase in neutrophil infiltration and pulmonary alveolar edema in the IR group compared to the control group. In a similar study, Kapan et al. [19] investigated erythropoietin's effects on lung IRI, uncovering escalated levels of pulmonary alveolar edema, interstitial edema, and inflammatory infiltration within the IR group relative to controls. In our study, the pathological changes, which we used as a reference during scoring the lung tissue injury, were evaluations of neutrophil infiltration, alveolar congestion, and interstitial edema similar to the studies found in the literature.

Edaravone, which we used in the treatment group in our study, is a powerful ROS scavenger and its main activity is through the inhibition of lipid peroxidation, which has an important role in the development of the IRI [22,23]. In addition, it inhibits the activation of phospholipase A2 production, thus decreasing lung edema and leukocyte extravasation [31]. Although there are experimental studies in the literature demonstrating the beneficial effects of edaravone on lung injury in IR models, we did not detect any studies designed as our model. Reyes et al. [21] investigated the effects of edaravone in the ischemia-reperfusion injury model in the isolated rat lung and determined that the values of lung injury scores were lower in the edaravone group compared to the IR group. In another study, Ito et al. [14] investigated the activity of edaravone in lung injury due to intestinal ischemia-reperfusion and determined that the lung injury score was significantly high in the IR group and significantly low in the edaravone group. In our ischemia-reperfusion model, the detectable lung injury rating was significantly higher in the IR group than in the sham group. The edaravone-administered group, on the other hand, showed a significant reduction in the score, indicating a statistically significant improvement.

In the context of our study's findings on the potential benefits of Edaravone in mitigating lung injury resulting from limb ischemia-reperfusion, it is essential to consider practical aspects that may influence its application in clinical settings. Edaravone, a novel scavenger of free oxygen radicals, has demonstrated promise in various experimental models, including ours. However, the availability and economic considerations associated with this agent are important factors to address. The availability of Edaravone can vary by region, and its cost-effectiveness in clinical practice may require careful evaluation. These practical considerations should be taken into account when translating our experimental results into clinical applications. As with many pharmacological agents, the feasibility and cost-effectiveness of utilizing Edaravone in patients undergoing vascular surgery or facing ischemia-reperfusion injuries deserve further investigation and clinical assessment.

CONCLUSION

Both through biochemical analysis and histopathological examination, it was substantiated that edaravone effectively reduced lung injury arising from lower limb ischemia-reperfusion. Drawing from its application in treating acute ischemic stroke across Japan and East Asian nations, there's a reasonable conjecture that this agent could similarly hold promise for addressing acute ischemia-reperfusion injury inherent to aortic or peripheral vascular surgeries. However, the full extent of its potential in such scenarios warrants further exploration through clinical investigations.

Ethics Committee Approval: This study was approved by the Committee on the Ethics of Animal Experiments of Gazi Osman Paşa University. (permit number: 51879863-06) Approval date: 08.01.2015.

Patient Consent for Publication: This study is a research using experimental animals.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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