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Original Article

Alpha B-crystallin Ameliorates Imbalance of Redox Homeostasis, Inflammation and Apoptosis in an Acute Lung Injury Model with Rats

Alpha B-kristalin Sıçan Akut Akciğer Hasarı Modelinde Redoks Homeostazının Dengesizliğini, Enflamasyonu ve Apoptozu İyileştirir

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ABSTRACT

Objective: Ischemia-reperfusion (IR) of the aorta is a significant contributor to the development of postoperative acute lung damage after abdominal aortic surgery. The aim of the present study was to examine the effect of alpha B-crystallin, a small heat shock protein (known as HspB5), on lung injury induced by abdominal aortic IR in rats.

Methods: Male Sprague-Dawley rats were divided into three groups: control, ischemia-reperfusion (IR, 90 min ischemia and 180 min reperfusion), and alpha B-crystallin +IR. Alpha B-crystallin (50 µg/100 g) was intraperitoneally administered 1 h before IR. Lung tissue samples were obtained for histological and biochemical analyses of oxidative stress and cytokine and apoptosis parameters in plasma, lung tissues, and bronchoalveolar lavage (BAL) fluid.

Results: The levels of malondialdehyde, reactive oxygen species, total oxidant status, tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), nuclear factor kappa B (NFKβ), caspase-9 (CASP-9), 8-hydroxy-2′-deoxyguanosine, total antioxidant status, superoxide dismutase, and interleukin-10 levels in lung tissues, plasma, and BAL fluid ($p<0.05$ versus control) increased in Aortic IR. However, alpha B-crystallin significantly reduced the lung tissue levels of oxidative, inflamatuvar, and apoptotic parameters in the plasma, lung tissues, and BAL fluid ($p<0.05$ versus aortic IR). Histopathological results showed that alpha B-crystallin ameliorated the morphological changes related to lung injury ($p<0.001$).

Conclusion: Alpha B-crystallin substantially restored disrupted the redox balance, inflammation, and apoptotic parameters in rats exposed to IR. The cytoprotective effect of alpha B-crystallin on redox balance might be attributed to improved lung injury.

Keywords: Abdominal surgery, cytokines, inflammation, immunology, ischemia-reperfusion

ÖZ

Amaç: İskemi-reperfüzyon (IR) aortası, abdominal aort cerrahisi sonrası akut akciğer hasarının önemli bir nedenidir. Çalışmamızın amacı, sıçanlarda abdominal aort IR tarafından indüklenen akciğer hasarı üzerine küçük ısı şok proteinlerinden biri olan alfa B-kristalinin (HspB5 olarak da bilinir) etkisini incelemektir.

Yöntemler: Erkek Sprague Dawley sıçanları üç gruba ayrıldı: kontrol, IR (IR, 90 dakika iskemi ve 180 dakika reperfüzyon) ve alfa B-kristalin+IR. Alfa B-kristalin (50 µg/100 g), IR'den bir saat önce intraperitoneal olarak verildi. Akciğer dokusu örnekleri histolojik analiz ve oksidatif stres parametreleri, sitokinler, apoptoz parametreleri açısından biyokimyasal analiz için alındı. Plazma, akciğer dokusu ve bronkoalveoler lavaj (BAL) sıvısında parametreler incelendi.

Bulgular: Aortik IR, akciğer dokularında malondialdehit, reaktif oksijen türleri, toplam oksidan durum, tümör nekroz faktörü-alfa (TNF-α), interleukin-1 beta (IL-1β), nükleer faktör kappa B (NFKβ), kaspaz-9 (CASP-9), 8-hidroksi-2′-deoksiguanozin, total antioksidan durum, süperoksit dismutaz, interleukin-10 seviyelerini anlamlı olarak artırırken ($p<0,05$, kontrol grubuna göre), alfa B-kristalin, plazma, akciğer dokusu ve BAL sıvısındaki oksidatif, enflamatuar ve apoptotik parametreleri anlamlı olarak azalmıştır ($p<0,05$, aortik IR'ye göre). Histopatolojik değerlendirme, alfa B-kristalinin akciğer hasarı ile ilişkili morfolojik değişiklikleri iyileştirdiğini göstermiştir ($p<0,001$).

Sonuçlar: Alfa B-kristalin, IR'ye maruz kalan sıçanlarda bozulmuş redoks dengesi, enflamasyon ve apoptotik parametreleri önemli ölçüde düzeltmiştir. Bu antioksidan etki, alfa B-kristalinin akciğer hasarı üzerindeki koruyucu etkisine bağlanabilir.

Anahtar Kelimeler: Abdominal cerrahi, sitokinler, enflamasyon, immünoloji, iskemi-reperfüzyon

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INTRODUCTION

Infrarenal abdominal aortic clamping and declamping after coronary artery surgery cause ischemia-reperfusion[1,2]. Distant organ damage is often associated with ischemia-reperfusion (IR) injury. The lungs are one of the distant organs that are damaged. IR inflammation exacerbates ischemic trauma and necessitates the maintenance of blood supply to prevent cell death[3]. Although the exact pathophysiological mechanisms of distant organ injury remain unclear, damage is attributed to oxidative, inflammatuvar, and apoptotic mediators released after IR[2,4].

Oxidative stress is an early and important symptom of acute lung injury. After reoxygenation of the lungs, a number of intricate events may take place, including oxidative damage to deoxyribonucleic acid (DNA) and proteins, peroxidation of membrane lipids, release of intracellular enzymes, increase in intracellular calcium, and neutrophil buildup in ischemic tissue[5,6]. Clinical and experimental studies have shown that IR in the lungs induces proinflammatory cytokines and apoptosis. In this condition, lung tissue are affected by oxidative, inflammatory, and apoptotic pathways[7,8]. The cause of lung injury as a result of IR following aortic surgery can be attributed to the release of cytokines, immune cells, various mediators secreted by immune cells, and their accumulation in the lungs[9]. Additionally, reactive oxygen species that cause DNA damage and apoptosis may lead to increased levels of proinflammatory cytokines and decreased expression of anti-inflammatory cytokines.

Heat shock proteins comprise a group of proteins including subfamilies. Their expressions are elevated under stress conditions. The subfamily includes small heat shock proteins. Alpha B-crystallin (known as HspB5) is one of the main lens proteins in vertebrates and a member of the small heat shock protein family[10]. The roles of alpha B-crystallin have been reported in different IR models. A retinal rat IR model showed that alpha B-crystallin decreased ischemic retinal damage, oxidative stress, iNOS, and nuclear factor kappa B (NFKβ) levels, and improved retinal function[11]. In addition, an experimental anterior ischemic optic neuropathy model in mice showed that both short-term treatment with alpha B-crystallin decreased inflammation and long-term treatment improved optic neuropathy function[12]. Kirbach et al.[13] demonstrated that HspB5 was upregulated in a rat cerebral ischemia model. In this study, HspB5 was one of the most significant HspBs in the neuronal stress response to IR injury. There are no articles in the literature about the expression of alpha B-crystallin after aortic IR-induced acute lung injury or its effect in a lung aortic IR model.

In addition, alpha B-crystallin has been used therapeutically for stroke and spinal cord contusion of mice studies[14,15]. In the first study, HspB5 decreased both the volume of stroke and inflammatory cytokines related to stroke pathology. In the second study, mice treated with HspB5 showed improvement of locomotor capabilities and a reduction in secondary tissue injury. In another study, the administration of alpha B-crystallin chronically improved cardiac function in a mouse myocardial infarction model. Mice treated with HspB5 exhibited increased left ventricular ejection fraction[16]. Different studies have shown that alpha B-crystalline decreases plasma interleukin (IL)-6[17], increases IL-10[18], inhibits NF-κB activation, suppress tumor necrosis factor-alpha (TNF-α) induced apoptosis[19], and reduces lipid peroxidation and the protective role of reactive oxygen against lung injury through its role in immune, inflammatory, and ischemic processes.

To our knowledge, no research has been conducted on the antioxidant, anti-inflammatory, and anti-apoptotic regulatory effects of alpha B-crystalline on aortic IR-induced lung damage *in vivo*. In the present study, we demonstrated that exogenous administration of alpha B-crystalline reduced lung tissue oxidative stress and cellular injury by inhibiting oxidative and proinflammatory responses, including cytokines.

MATERIALS and METHODS

Animals

Adult 24 male Sprague-Dawley rats (12 weeks-average weight: 320 grams) were housed in separate cages in a standardized temperature and light-dark cycle controlled environment. Animals had free access to standard feed and water. The animal studies were conducted after receiving approval from the Institutional Animal Care and Istanbul University Animal Experiments Local Ethics Committee (decision no: 2014/62 date: 29.05.2014).

Sprague-Dawley rats were divided into three groups. 1) Control (C; n=8) group: the control group underwent midline laparotomy and dissection of infrarenal abdominal aorta (IAA) without occlusion. 10 mL physiological serum was administered to this group into the peritoneal cavity during the procedure. IR procedure was not applied. 2) Ischemia-Reperfusion (IR; n=8) group: 10 mL physiological serum was administered into the peritoneal cavity during the procedure. In addition, 90 min of ischemia and 180 min of reperfusion were performed. 3) Alpha B-crystallin+Ischemia-Reperfusion (Alpha B-crystallin+IR; n=8) group: 10 mL physiological serum was administered during the procedure. Intraperitoneal

alpha B-crystallin administration (50 µg /100 g) were performed for 1 h before ischemia, 90 min of ischemia, and 180 min of reperfusion. We used the dosage of alpha B-crystallin according to its nontoxic effects[18,20].

Ischemia-reperfusion Procedure

All animals were anesthetized intraperitoneally with thiopental sodium (60 mg/kg). Tracheotomy was performed after anesthesia; trachea was intubated with a polyvinylchloride cannula. Animals were placed on a heating plate to maintain body temperature at 37 ˚C during experiment. A 22-gauge catheter was inserted to draw blood from the carotid artery and monitor blood pressure. After the skin of rats was prepared aseptically, a midline laparotomy was performed, and the intestines were pulled to the left with wet gauze. Heparin was mixed with physiological serum 50 U/kg (500 mL volume), (Nevpar's; MN Pharmaceutical, Istanbul, Türkiye) before the ischemia protocol to prevent clotting. The area called the IAA just below the right and left kidney arteries was isolated, and dissection of IAA was performed in the IR and alpha B-crystallin+IR groups. A non-traumatic microvascular clamp (vascu-statts II, midi straight 1001-532; Scanlan Int, St Paul, MN) was placed to perform the 90 min ischemia protocol. 10 mL of physiological serum adjusted to body temperature was administered to the peritoneal cavity. After suturing the incision area with surgical thread, the area was covered with moist gauze to minimize fluid and heat loss. After 90 min of ischemia, the incision was reopened, and the microvascular clamp from the infrarenal aortic area was removed[21]. These changes were also confirmed using a computerized system (PowerLab, 16 SP, ADInstruments, Castle Hill, Australia) with systemic arterial blood pressure records from the carotid artery. At the end of 180 min of reperfusion, a high dose of intravenous thiopental sodium (150 mg/kg) was administered for euthanasia after collecting bronchoalveolar lavage (BAL) fluid samples from the lungs and blood from the carotid artery. The lung was removed together with the trachea, and the lower right lung lobe was stored in the formol for histological examination. The lung, BAL fluid, and plasma samples were stored at -80 ˚C for biochemical analyses.

Bronchoalveolar Lavage (BAL) Procedure

The left main bronchus was cannulated and secured. Saline (5 mL) was injected into three quick aliquots (5 mL) each, with each aliquot withdrawn slowly. The BAL specimen was collected with a fluid recovery of 90% or greater[22]. The fluid was centrifuged at 1500 rpm for 8 minutes at +4 ˚C. The supernatant was then stored at -80 ˚C until biochemical analysis.

Lung Tissue, Plasma, and BAL Fluid Biochemical Analysis

Lung tissue samples were washed in physiological serum and phosphate buffer and then crushed by freezing in liquid nitrogen. After thawing, phosphate buffer was added, and the tissues were homogenized. Homogenates were centrifuged (3000 rpm, 20 min, +4 ˚C), and the resulting supernatants were used for biochemical analysis. BAL fluid and plasma samples were collected after reperfusion. Levels of malondialdehyde (MDA), reactive oxygen species (ROS), total oxidant status (TOS), total antioxidant status (TAS), superoxide dismutase (SOD), IL-10, TNF-α, IL-1β, NF-κB, CASP-9, and 8-OHdG were quantified using Sunred ELISA kits.

Histological Evaluation

The middle lobe of the right lung was fixed in 10% neutral formalin, embedded in paraffin, and sectioned into 5 µm slices. After deparaffinization and staining with hematoxylin and eosin, the sections were evaluated histologically by two independent pathologists using a light microscope (Olympus BX61, Japan). Histologic injury scores were determined based on five parameters: alveolar wall thickness, intra-alveolar edema-infiltration, intra-alveolar hemorrhage, capillary blood collection, and alveolar damage, each scored on a scale from 0 to 4 (0: absent and normal appearance, 1: mild, 2: moderate, 3: severe, 4: intense). Additionally, the total lung injury score was calculated for each animal group[23].

Statistical Analysis

The data was processed by the statistical analysis software GraphPad Prism version 5.0 for Windows. The Bonferroni test was used to assess significant variations between groups using one-way analysis of variance (ANOVA). Statistical significance was set as $p<0.05$. The histological data was processed by the statistical analysis software Sigma Stat for Windows, version 3.0 (Jandel Scientific, San Rafael, CA) and one-way ANOVA. The Holm-Sidak test was used to assess group variations. Statistical significance was set as $p\leq0.001$. All the data are shown as the mean values±standard deviation.

RESULTS

Oxidative Stress Parameters

When the ELISA results for oxidative stress markers were analyzed in all animal groups, lung tissue, BAL, and plasma results were similar. ROS, TOS, and MDA levels increased in the IR group compared with the control group. Alpha B-crystallin application decreased these parameters in alpha B-crystallin+IR group compared

to IR group. Table 1 shows the p-values, which are the statistically significant differences between means of groups. SOD and TAS values increased in the alpha B-crystallin+IR group compared with the IR group (Figures 1 and 2).

Inflammation Parameters

When inflammation parameters in all animal groups were compared, TNF-α, IL-1β and NF-κB levels differed between the IR and control groups. TNF-α, IL-1β and NF-κB were higher in the IR group. Compared with the

Table 1. Oxidative stress marker levels in lung tissue, plasma, and bronchoalveolar lavage fluid of all groups.

		Control (n=8)	Ischemia-reperfusion (n=8)	Alpha B-crystallin+ischemia reperfusion (n=8)	p-value
MDA	Tissue (nM/mg)	15.31±0.86	26.12±1.39^{a***}	18.54±0.71^{b***}	^{a}p<0.001 ^{b}p<0.001
	Plasma (nmol/mL)	9.94±0.38	14.90±0.73^{a***}	10.70±0.44^{b***}	^{a}p=0.001 ^{b}p=0.004
	BAL fluid (nmol/mL)	14.68±0.21	27.99±1.69^{a***}	15.45±0.44^{b***}	^{a}p=0.000 ^{b}p=0.000
ROS	Tissue (U/mg)	3905,66±211.35	5540,10±234.76^{a***}	4496,93±221.86^{b**}	^{a}p<0.001 ^{b}p<0.001
	Plasma (U/mL)	1919,61±201.83	2842,11±112.01^{a**}	1989.91±152.94^{b**}	^{a}p=0.008 ^{b}p=0.006
	BAL fluid (U/mL)	2721,36±50.21	3866,93±103.60^{a***}	2986,14±241.18^{b**}	^{a}p=0.002 ^{b}p=0.004
TOS	Tissue (nM/mg)	5.21±0.41	7.70±0.34^{a***}	5.62±0.30^{b**}	^{a}p<0.001 ^{b}p<0.001
	Plasma (nM/mL)	3.17±0.09	4.13±0.17^{a***}	3.33±0.17^{b**}	^{a}p=0.005 ^{b}p=0.012
	BAL fluid (nM/mL)	4.07±0.16	6.66±0.54^{a***}	4.66±0.16^{b**}	^{a}p=0.001 ^{b}p=0.012

Data are expressed as mean±standard deviation. *p<0.05, **p<0.01, ***p<0.001, ^{a}: Comparisons between the control and IR groups, ^{b}: Comparisons between the IR and alpha B-crystallin+IR groups, MDA: Malondialdehyde, ROS: Reactive oxygen species TOS: Total oxidant status BAL: Bronchoalveolar lavage

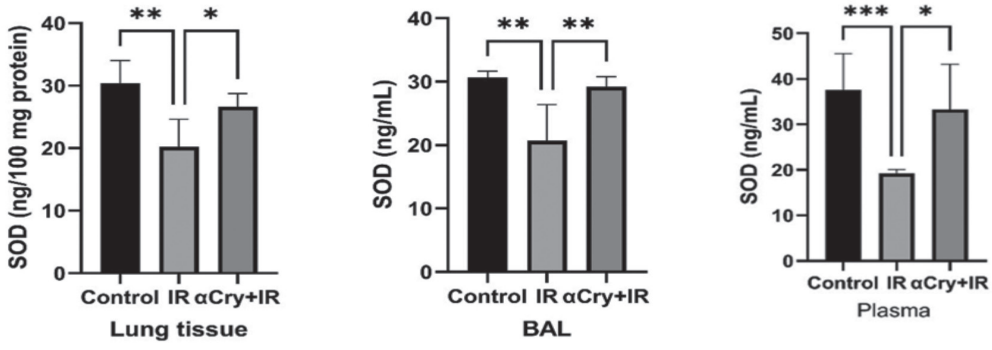

Figure 1. Lung tissue, BAL, and plasma SOD levels of groups. Experimental groups: Control, IR, IR administered alpha B-Crystallin (aCry+IR) group.

*p<0.05, **p<0.01, ***p<0.001, BAL: Bronchoalveolar lavage, SOD: Superoxide dismutase, IR: Ischemia-reperfusion

IR group, the levels of these parameters in the alpha B-crystallin+IR group decreased. Table 2 shows the means of groups and p-values. When we looked at the IL-10 levels, IL-10 was significantly decreased in the IR group. However, when the IR and alpha B-crystallin+IR groups were compared, IL-10 levels increased significantly in the alpha B-crystallin+IR group (Figure 3).

Apoptotic Parameters

When we analyzed apoptotic parameters in the rat lung and plasma of all groups, CASP-9 and 8-OHdG levels were significantly higher in the IR group than in the control group. Alpha B-crystallin application reduced these parameters in alpha B-crystallin+IR group

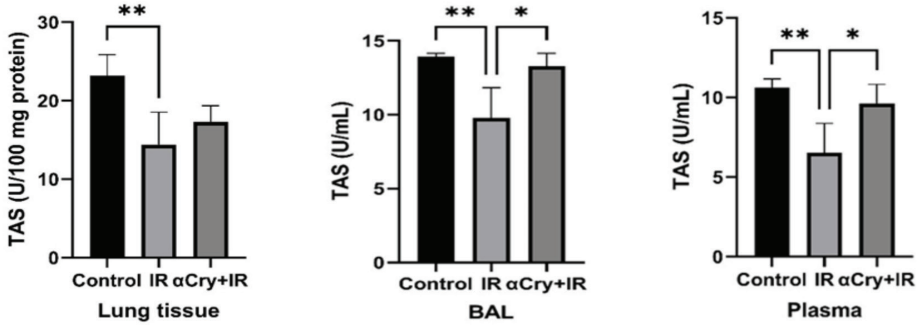

Figure 2. Lung tissue, BAL, and plasma TAS levels of groups. Experimental groups: Control, ischemia-reperfusion (IR), IR administered alpha B-Crystallin (aCry+IR) group.

Control, ischemia-reperfusion (IR), IR administered alpha B-Crystallin (aCry+IR) group.

TAS: Total Antioxidant Capacity, BAL: Bronchoalveolar lavage,*p< 0.05 **p< 0.001,

Table 2. Inflammation marker levels in lung tissue, plasma, and bronchoalveolar lavage fluid of the groups.

		Control (n=8)	Ischemia-Reperfusion (n=8)	Alpha B-crystallin+ Ischemia-Reperfusion (n=8)	p-value
TNF-α	Tissue (ng/mg)	269.81±15.10	422.49±14.74^{a***}	321.27±5.18^{b**}	^{a}p=0.000 ^{b}p=0.045
	Plasma (ng/L)	152.55±7.94	269.20±14.55^{a***}	175.26±4.85^{b***}	^{a}p=0.002 ^{b}p=0.001
	BAL fluid (ng/L)	192.23±6.22	294.55±4.88^{a***}	219.64±19.33^{b***}	^{a}p<0.0001 ^{b}p=0.012
IL-1β	Tissue (pg/mg)	2101,22±185.70	2887,22±150.56^{a**}	2247.28±53.82^{b*}	^{a}p=0.032 ^{b}p=0.033
	Plasma (pg/L)	1127,82±19.71	1514,79±46.80^{a***}	1181.54±33.03^{b***}	^{a}p=0.000 ^{b}p=0.004
	BAL fluid (pg/L)	1795,68±19.05	2513,64±129.67^{a***}	1941,38±36.79^{b***}	^{a}p=0.002 ^{b}p=0.007
NF-κB	Tissue (ng/mg)	5.23±0.38	9.31±0.56^{a***}	6.77±0.38^{b**}	^{a}p=0.000 ^{b}p=0.012
	Plasma (ng/mL)	3.07±0.19	5.11±0.28^{a***}	3.59±0.14^{b***}	^{a}p=0.000 ^{b}p=0.003
	BAL fluid (ng/mL)	4.54±0.10	6.05±0.09^{a***}	5.04±0.31	^{a}p=0.000

Control group, IR Ischemia-Reperfusion group, Alpha B-crystallin+IR Alpha B-crystallin+Ischemia-Reperfusion group. Data are expressed as mean±standard deviation. *p<0.05, **p<0.01, ***p<0.001, ^{a}: comparisons between the Control and IR group, ^{b}: comparisons between the IR and Alpha B-crystallin+IR group, TNF-α: Tumor necrosis factor-alpha, IL-1β: interleukin 1 beta, NF-κβ: Nuclear factor kappa beta BAL: Bronchoalveolar lavage

compared to IR group. Figures 4 and 5 show the CASP-9 and 8-OHdG parameters in the specimens.

Histopathologic Results

Histopathological examination of the right lung lobe was performed in all animal groups. Total lung injury was the least in the control group and the most severe IR group. The lung injury score was significantly higher in the IR group (9.0±0.8 p<0.001) than in the control group (2.286±0.714 p<0.001). Lung injury parameters in the IR group included intra-alveolar macrophage, neutrophil infiltration, alveolar damage, and alveolar wall thickening. Application of alpha B-crystallin before IR significantly reduced IR damage, and the total lung injury score decreased significantly in the alpha B-crystallin+IR group [5.56±0.56 p<0.001] compared with the IR group (9.0±0.8 p<0.001). Lung injury parameters observed in the alpha B-crystallin+IR group were less evident (Figure 6).

DISCUSSION

Our study showed that 90 min of infrarenal aortic occlusion followed by 180 min of reperfusion resulted in acute lung damage, including destruction of the alveolar architecture and inflammatory cell infiltration. The levels of MDA, ROS, TOS, TNF-α, IL-1β, NF-κB,, CASP-9, and 8-OHdG increased, whereas the levels of SOD, TAS, and IL-10 decreased in plasma, BAL fluid, and lung tissue samples. Interestingly, pre-administration of alpha B-crystallin 1 h before ischemia mitigated these effects, improving oxidative, inflammatory, and apoptotic

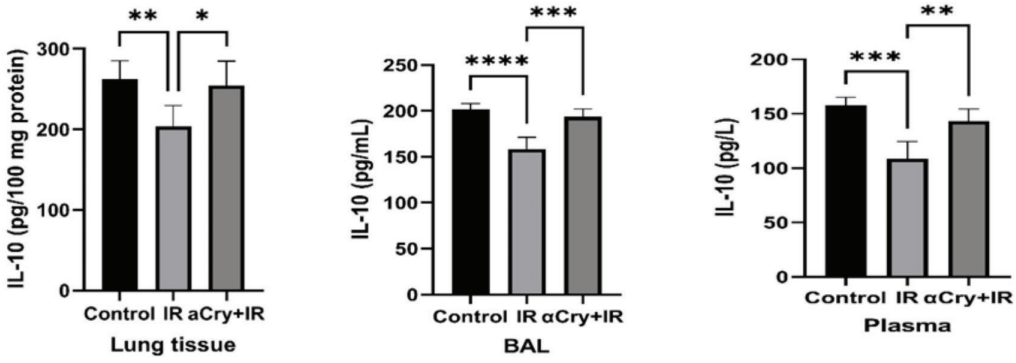

Figure 3. IL-10 Lung tissue, BAL, and plasma IL-10 levels of groups. Experimental groups: control, IR administered alpha B-Crystallin (aCry+IR) group.

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, IL-10: interleukin, BAL: Bronchoalveolar lavage IR: Ischemia-reperfusion

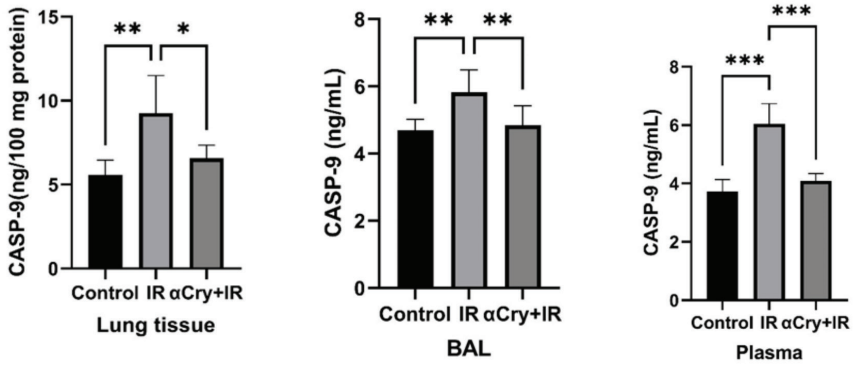

Figure 4. Lung tissue, BAL, and plasma CASP-9 levels of groups. Experimental groups: control, IR administered alpha B-Crystallin (aCry+IR) group.

*p< 0.05 , **p< 0.01, ***p< 0,001, CASP-9: caspase-9, BAL: Bronchoalveolar lavage, IR: Ischemia-reperfusion

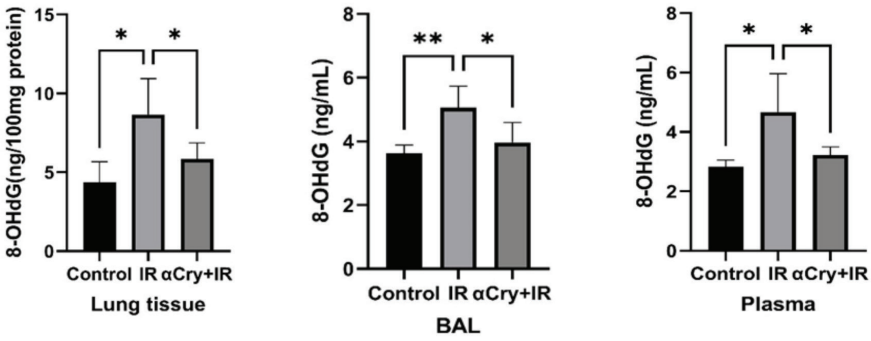

Figure 5. 8-OHdG Lung tissue, BAL, and 8-OHdG plasma levels of groups. Experimental groups: control, IR administered alpha B-Crystallin (aCry+IR) group.

*p< 0.05, **p< 0.01, 8-OHdG: 8-hydroxy-2′ -deoxyguanosine, BAL: Bronchoalveolar lavage, IR: Ischemia-reperfusion

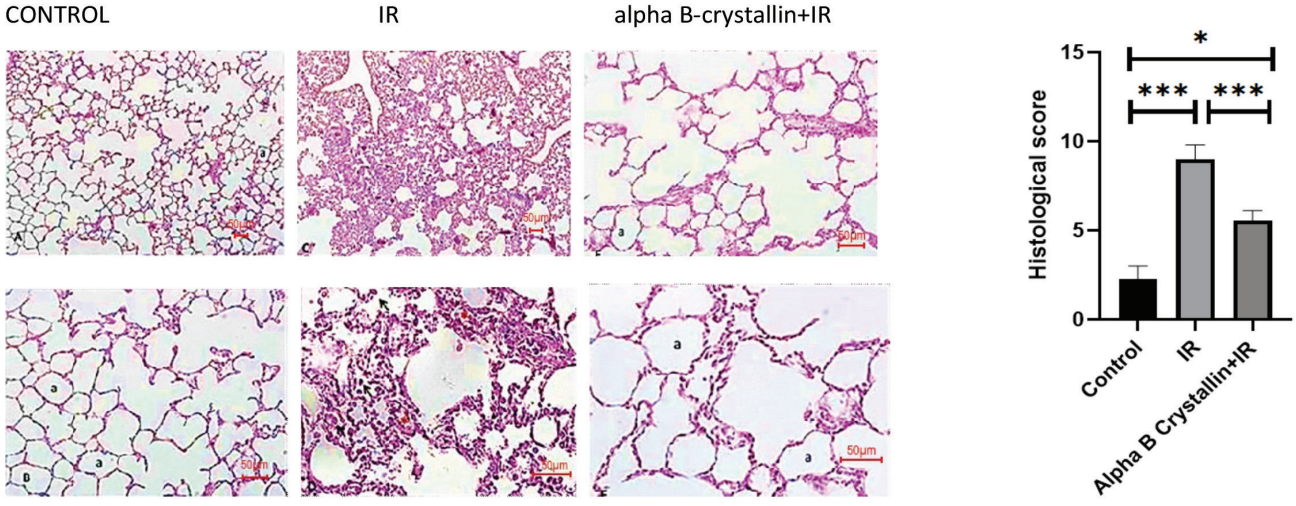

Figure 6. Histological sections of lung tissues from different experimental groups: control **(A, B)**, IR **(C, D)**, and alpha B-crystallin+IR **(E, F)** groups; histological scoring graph. In panels A and B, minimal histological changes with thin alveolar walls are observed. Panels C and D exhibit significant lung damage characterized by intra-alveolar infiltration (black arrow) and alveolar damage. Panels E and F, in contrast, show mild alveolar damage. The effects of alpha B-crystallin on histopathological changes in the lung tissue of rats with IR-induced acute lung injury are shown in panels E and F. Statistical significance is denoted as *p< 0.05, ***p< 0,001. (H&E A and C x10, B and E x20, D and F x40), IR: Ischemia-reperfusion

parameters in plasma, lung tissue, and BAL fluid samples and enhancing cellular structure.

Lung injury, IR, and systemic inflammation are clinically interrelated. Physiopathological mechanisms describing the interaction of these three titles are not fully understood. Various mediators are released by immune cells after IR injury, and these mediators seem to be important in the development of pulmonary damage[24]. Increase in biochemically triggered cytokine levels after aortic aneurysm treatment causes death with fever, tissue destruction, and even septic shock postoperatively[2].

Aortic IR injury causes pulmonary dysfunction[21]. IR injury is the result of a sequence of biochemical processes in which unbalanced ROS scavenging[25]. Increased ROS

levels can lead to protein, DNA, and lipid peroxidation, as well as mitochondria-induced cell death, which can contribute to IR injury[26]. One of the related therapies for IR injury is antioxidant therapy. Antioxidants have been shown to reduce cellular and tissue damage by lowering intracellular ROS levels and reducing oxidative stress[27].

Tural et al.[28] determined that aortic occlusion contributes to the increase in lung tissue MDA levels. Similarly, we observed that aortic occlusion increased pulmonary tissue MDA levels. There was a significant positive correlation between reactive oxygen species and MDA, a marker of lipid peroxidation. Further analysis showed that the application of alpha B-crystallin before IR decreased the production of reactive oxygen species and lipid peroxidation. It was also found that the SOD and TAS parameters increased. This implies that alpha B-crystallin systematically exerts an antioxidant effect.

Changes in the processes following IR can cause pulmonary apoptosis. Morphologically, apoptosis can also be characterized by DNA fragmentation[29]. Caspase-9 and 8-OhDG analyses were performed to demonstrate apoptosis and DNA damage. According to our results, the value of these parameters increased significantly not only in the lungs but also in plasma and BAL fluid in the IR group. 8-OHdG is the dominant form of oxidative damage caused by free radical species in nuclear and mitochondrial DNA and is used as a biomarker for oxidative stress[30]. Increased 8-OHdG levels of oxidative stress in the aortic IR-induced lung injury group indicates nuclear or mitochondrial DNA damage occurred. A significant reduction in 8-OHdG was detected in all samples from the alpha B-crystallin-treated group compared with the IR group. In addition, a significant decrease in caspase-9 expression was observed following alpha B-crystallin treatment. Initial studies have shown that alpha B-crystallin is effective against proapoptotic proteins and stops the translocation of Bax and Bcl-Xs[31]. Another anti-apoptotic role of alpha B-crystallin is its ability to prevent caspase-3 activation regardless of intrinsic or extrinsic pathway[32,33]. In light of this information, we can state that alpha B-crystallin affects apoptosis through different mechanisms and prevents apoptosis by inhibiting caspases. The effect of alpha B-crystallin on caspase-9 levels probably occurs via two mechanisms. One of the possible explanations for this is that alpha B-crystallin connects Bax and prevents the transfer of Bax from mitochondrial membrane from the cytosol[32]. Kamradt et al.[34] confirmed that alpha B-crystallin disrupts the proteolytic activation of caspase-3. In light of this information, another possible explanation is that alpha B-crystallin prevents the transition of procaspase-9 from an inactive to an active form, caspase-9. The secondary pathway can be more possible due to the proteolytic activation of caspase 9.

Histopathological examination of lung tissues in the control group showed less severe disease than that in the IR group. Intra-alveolar macrophages, neutrophil infiltration, alveolar damage, and alveolar wall thickening were observed in the lung tissues of the IR group. These injuries were less evident in rats administered alpha B-crystallin than in the IR group. Our analysis found that in rats treated with alpha B-crystallin, total histological injury was substantially reduced. Different studies showed that TNF-α and IL-1β play an important role in IR damage. These proinflammatory cytokines damage vascular beds, activate neutrophils, and migrate to the alveolar area[35]. Activated neutrophils release free radicals, proteolytic enzymes and peroxidase. Activated neutrophils can also lead to acute lung injury by increasing pulmonary vascular permeability[36].

Our experiments showed that TNF-α and IL-1β levels increased, whereas IL-10 levels decreased, in rats exposed to IR. This decrease may have resulted from the suppression of activity of IL-10 by TNF-α and IL-1β [37]. The transcription factor NF-κB, which promotes the expression of proinflammatory cytokines such as TNF-α and IL-1β, is also linked to neutrophil infiltration into the lungs and lipid peroxidation[38,39]. In our study, the increase of TNF-α and IL-1β together with NF-κβ suggests that inflammation in acute lung injury is increased by NF-κβ-mediated proinflammatory cytokines. Based on our results of decreasing TNF-α and IL-1β levels and increasing IL-10 levels in rats, we conclude that alpha B-crystallin reduces acute inflammation. This effect of alpha B-crystallin may inhibit the activity of proinflammatory cytokines by inhibiting the suppression of IL-10. Levels of NF-κβ decreased in all alpha B-crystallin+IR groups compared to IR group. Lentsch et al.[40] showed that the inflammatory-suppressing effect of IL-10 occurs by inhibiting the translocation of NF-κβ from cytoplasm to the nucleus in rat lung tissues. However, this study has some limitations. First, the unavailability of resources for immunohistochemical staining may limit the representation of apoptotic DNA fragmentation in TUNEL assays. Second, western blotting could be used to identify proteins, but insufficient resources limits this.

CONCLUSION

Based on our findings, alpha B-crystallin has the potential to prevent acute lung injury caused by aortic IR, mainly because of its antioxidant and anti-inflammatory properties. It also appears to mitigate DNA damage and

cell apoptosis associated with IR. Preadministration of alpha B-crystallin before aortic surgery could potentially mitigate IR injury. The present study underscores the potential of alpha B-crystallin as a novel treatment for IR-induced lung injury, marking its debut in this therapeutic context.

Ethics

Ethics Committee Approval: Approval was received from the Istanbul University Animal Experiments Local Ethics Committee (decision no: 2014/62 date: 29.05.2014).

Informed Consent: Because this article is based on animal studies, patient consent is not required.

Author Contributions

Surgical and Medical Practices: S.K., I.G., M.O.Y., G.S., Concept: S.K., I.G., G.S., Design: S.K., I.G., G.S., Data Collection and/or Processing: S.K., I.G., M.O.Y., T.E.Y., E.E.G.M., N.Y., G.S., Analysis and/or Interpretation: S.K., I.G., M.O.Y., T.E.Y., E.E.G.M., N.Y., G.S., Literature Search: S.K., I.G., E.E.G.M., G.S., Writing: S.K., I.G., E.E.G.M., G.S.

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