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Effect of IL-18 binding protein on hepatic ischemia-reperfusion injury induced by infrarenal aortic occlusion

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Purpose: Severe local and systemic tissue damage called ischemia/reperfusion (IR) injury occurs during the period of reperfusion. Free oxygen radicals and proinflammatory cytokines are responsible for reperfusion injury. IL-18 binding protein (IL-18BP) is a natural inhibitor of IL-18. The balance between IL-18 and IL-18BP has an important role in the inflammatory setting. The present study aimed to investigate whether IL-18BP had a protective role in remote organ hepatic IR injury.
Methods: Wistar-Albino rats were divided into three groups that contained seven rats. Group I (sham): Laparotomy and infrarenal abdominal aorta (AA) dissection were done but no clamping was done. Group II (I/R): The infrarenal AA was clamped by atraumatic microvascular clamp for 30 minutes and then was exposed to 90 minutes of reperfusion. Group III (IR + IL-18BP): 75 μg/kg of IL-18BP in 0.9% saline (1 mL) was administered 30 minutes before infrarenal AA dissection and clamping; 30 minutes of ischemia was applied and then was exposed to 90 minutes of reperfusion.
Results: Serum AST, ALT, and LDH levels were remarkably higher in IR group and returned to normal levels in treatment group. The proinflammatory cytokine levels had decreased in treatment group, and was statistically significant compared with the IR group. Serum levels of total oxidant status and oxidative stress index decreased and levels of total antioxidant status increased by IL-18BP.
Conclusion: This study suggested that IL-18BP has antioxidant, anti-inflammatory and hepatoprotective effects in cases of IR with infrarenal AA induced liver oxidative damage.
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Key Words: Interleukin-18, IL-18 binding protein, Reperfusion Injury, Transplantation, Acute liver injury

INTRODUCTION

Restoration of blood supply is the main factor for ischemic organs to maintain their vitality. As first described by Haimovici [1] in 1960, severe local and systemic tissue damage occurs during the period of reperfusion when tissue oxygenation is restored. This damage is called ischemia/reperfusion (IR) injury. Free oxygen radicals, as well as proinflammatory cytokines, are responsible for reperfusion injury. IR injury occurring after aortic clamping, which is a part of transplantation, trauma, and major vascular surgery, is observed not only in the lower extremities, but also in the distant organs and tissues such as the lungs, kidneys, heart, and liver [2]. Although many circulating chemical mediators and potential microembolisms

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play a role in infrarenal aortic IR, the washout phenomenon is the most common one considered to be responsible [3]. IL-18, a proinflammatory cytokine is produced by macrophages, epithelial cells, and T-cells, has an active role in inflammatory incidents and the immune system. It is known that damage due to IL-18 is independent from the neutrophils and CD-4 T cells [4]. IL-18, which plays an active role in inflammatory incidents, such as IR, is mainly responsible for producing TNF and IL-1 from mononuclear cells. IL-18 is also involved in inflammatory reactions by modulating the activity of macrophages. IL-18 binding protein (IL-18BP) is a natural inhibitor of IL-18. The balance between IL-18 and IL-18BP has an important role in the inflammatory setting. IL-18BP was used as a therapeutic agent in some studies [5]. It was demonstrated that the combined IL-18BP-Fc successfully neutralized IL-18 and had a protective effect against the Fas/FasL-induced damage [6]. There are no studies on the protective effect of IL-18BP in hepatic IR injury induced by aortic clamping that are frequently encountered in routine practices such as transplantation, shock, and major abdominal surgery. The present study aimed to investigate whether IL-18BP had an anti-inflammatory, antioxidant, or protective role in the distant organ hepatic IR injury secondary to infrarenal aortic clamping through histopathological, immunohistochemical and biochemical methods.

METHODS

The study protocols and experimental methods were approved by the local Institutional Ethics Committee of Experimental Animals in the University of Afyon Kocatepe. Care of all rats was done according to the Experimental Animal Usage and Principles regulated by the National Health and Medical Research Council and according to the Guide for Experimental Animal Care and Usage prepared and issued by the National Institution of Health. In this study, a total of 21 male Wistar-Albino rats weighing 250–300 g (mean, 270 g) were randomly divided to three groups comprising 7 rats in all groups. The three groups of seven rats were as follows:

Group I (sham, n=7): Laparotomy and infrarenal abdominal aorta (AA) dissections were done with the same surgical duration and stress as in other groups but no clamping was done to infrarenal AA.

Group II (I/R, n=7): About 1-mL 0.9% saline was administered intraperitoneally 30 minutes before and after occlusion. After laparotomy and dissection of infrarenal AA, infrarenal AA was clamped by atraumatic microvascular clamp for 30 minutes and then was exposed to 90 minutes of reperfusion.

Group III (IR + IL-18BP, n=7): 75 μg/kg of IL-18BP in 0.9% saline (1 mL) was administered intraperitoneally 30 minutes before infrarenal AA dissection and clamping, followed by 30 minutes of applied ischemia and then exposed to 90 minutes of reperfusion.

Substance preparation

Human and murine IL-18BP isoforms are active across species [5]; therefore, we used recombinant human IL-18BP (119-BP, R&D Systems, Minneapolis, MN, USA) in this study that was administered intraperitoneally 30 minutes before infrarenal AA dissection and clamping.

Anesthesia and surgical procedure

General anesthesia was induced by 5 mg/kg intramuscular xylazine (Rompun, Bayer, Istanbul, Turkey) and 40-mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, Eczacibasi, Turkey). Animals were placed in a supine position and median laparotomy was applied to rats under sterile conditions. After bowels were moved away by wet surgical gauze, infrarenal AA were carefully explored. Following 150 U/kg of heparin administration, the infrarenal AA was clamped for 30 minutes by atraumatic microvascular clamp (SCANLAN Vascu-Statt, Single-Use Bulldog Clamps, SCANLAN, Saint Paul, MN, USA). Aortic ischemia was confirmed by loss of pulsation on distal aorta and aortic reperfusion was confirmed by return of pulsation on distal aorta after removal of clamp. An abdominal incision was temporarily covered up by a plastic clothing to minimize loss of heat and fluid. A following 10 mL of saline solution was administered intraperitoneally to preserve fluid balance. After 30 minutes, the abdomen was explored again and clamped on and 90 minutes of reperfusion was applied. After the period of reperfusion (90 minutes), all rats were killed by decapitation. Before the rats were killed, 3 mL of blood samples were collected from by heart puncture for biochemical examinations, and centrifuged to obtain serum. Serum samples were stored at –70°C for biochemical examinations. In addition, livers were removed for investigation.

Biochemical analyses

Serum AST, ALT, and LDH levels were indicators of liver ischemia, which were analyzed in an autoanalyzer (Cobas 6000, Roche, Basel, Switzerland). Serum IL-1β, IL-6, TNF-α, IFN-γ (E-Bioscience, Vienna, Austria) and IL-18 (Booster, Fremont, CA, USA) levels were determined by ELISA technique using specific kits, and the results were expressed as pg/mL.

Measurement of serum total oxidant status

The total oxidant capacity (TOC) of the serum was measured using an automated colorimetric measurement method for TOC [7]. In this method, oxidants presented in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules, which are abundantly presented in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an

acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules presented in the sample. The assay was calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (μmol H_2O_2 Eq/L).

Measurement of serum total antioxidant status

The total antioxidant capacity (TAC) of the serum was measured using a novel automated colorimetric measurement method for TAC [8]. This method is based on the bleaching of color characteristics of a more stable ABTS (2,2'-azino-bis[3-ethylbenzothiazo-line-6-sulfonic acid]) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L.

Determination of serum oxidative stress index

The ratio of serum oxidative stress (TOS) to tissue antioxidant status (TAS) represents the oxidative stress index (OSI), an indicator of the degree of oxidative stress (OS). The OSI value is calculated according to the formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L)/TAS (mmol Trolox equivalent/L) × 100 [9].

Histopathological and immunohistochemical examination

Tissue samples were fixed in 10% neutral formalin solution, histologically processed and embedded in paraffin blocks. Sections were taken from these blocks at 5-μ thickness and were mounted on both normal and poly-l-lyzine coated slides. While the normal slides were stained with hematoxylin-eosin dye for general morphological evaluation, poly-l-lyzine coated slides were used for immunohistochemical staining. Stained slides were then evaluated under light microscope (Eclipse E-600, Nikon, Tokyo, Japan). For the evaluation of Hematoxylin Eosin stained slides, inflammatory cell migration, edema and sinusoidal enlargement were semiquantitatively evaluated and scored from 0 to 4 parallel to their degree. On the other hand, in the evaluation of inducible nitric oxide synthase (iNOS)expression an immunohistochemical score was used. The immunoreactivity of iNOS positive cells in 6 different areas under ×40 objective magnification for each slide were calculated and used for statistical analysis.

Statistical analyses

The data were analyzed by using SOFA Statistics ver. 1.4.2 open source software for Windows. Results for descriptive statistics were expressed as mean ± standard deviation or median (range). Statistical comparisons of continuous variables among the groups were performed using one-way analysis of variance or Kruskal-Wallis test based on their distribution. Tukey test was performed for post hoc analysis after performing analysis of variance test. In cases where Kruskal-Wallis test yielded statistical significance, Bonferroni-corrected Mann-Whitney U test was used to identify the groups which showed differences. A P-value of <0.05 was considered statistically significant.

Table 1. Serum levels of inflammatory markers and oxidative stress markers

Variable	Group			P-value
	Sham	IR	IR + IL-18BP	
AST (U/L)	177.3 (153.7–195.9)[a,c]	555.3 (355.3–747)[a,b]	353.3 (226.4–637)[b,c]	<0.001*,[e]
ALT (U/L)	50.2 (36.9–69.2)[a,c]	114.9 (63.9–182.3)[a,b]	66.1 (58.8–78.5)[b,c]	0.003*,[e]
LDH (U/L)	2,436.85 ± 707.1[a]	3,423 ± 410.96[a]	2,928.57 ± 755.32	0.034*,[d]
IFNγ (pg/mL)	58.66 ± 5.23[a]	78.61 ± 8.35[a,b]	64.98 ± 6.58[b]	<0.001*,[d]
IL-1β (pg/mL)	39.93 ± 4.24[a]	47.72 ± 7.63[a]	43.65 ± 3.90	0.053*,[d]
IL- 6 (pg/mL)	99.92 ± 2.32[a]	107.86 ± 4.76[a,b]	100.29 ± 6.1[b]	0.008*,[d]
IL-18 (ng/mL)	170.73 ± 6.82[a]	190.3 ± 14.3[a,b]	175.87 ± 8.19[b]	0.006*,[d]
TNF-α (pg/mL)	130.94 ± 6.57[a]	142.74 ± 6.92[a,b]	133.67 ± 5.53[b]	0.007*,[d]
TOC (mmol H_2O_2 Eq/L)	4.99 (3.9–6.33)[a]	7.85 (7.08–8.42)[a,b]	4.95 (4.45–5.92)[b]	<0.001*,[e]
TAC (mmol Trolox equivalent /L)	1.38 ± 0.34	1.15 ± 0.17	1.32 ± 0.56	0.555[d]
OSI	404.59 ± 190.37[a]	695.28.1 ± 139.02[a,b]	439.03 ± 165.5[b]	0.008*,[d]

Values are presented as median (range) or mean ± standard deviation.
IR, ischemia/reperfusion; IL-18BP, IL-18 binding protein; TOC, total oxidant capacity; TAC, total antioxidant capacity; OSI, oxidative stress index.
[a]Compared between Sham and IR group. [b]Compared between IR and IR + IL-18Bp group. [c]Compared between Sham and IL-18Bp group. [d]One-way analysis of variance and post hoc analysis with Tukey test. [e]Kruskal-Wallis test and post hoc analysis with Bonferroni-corrected Mann-Whitney U test. *P < 0.05, significant difference.

RESULTS

Assessment of biochemical data on hepatic damage

The blood level of AST was found to be 177.3 U/L (range, 153.7–195.9 U/L) in the sham group, 555.3 U/L (355.3–747 U/L) in the IR group, and 353.3 U/L (226.4–637 U/L) in the IL-18BP group. There was a statistically significant difference between the IR group and sham group, and the IL-18BP group and IR group ($P = 0.00$). The blood level of ALT was found to be 50.2 U/L (36.9–69.2 U/L) in the sham group, 114.9 U/L (63.9–182.3 U/L) in the IR group, and 66.1 U/L (58.8–78.5 U/L) in the IL-18BP group. There was a statistically significant difference between the IR group and sham group, and the IL-18BP group and IR group ($P = 0.03$). The blood level of LDH was 2,436.85 ± 707.1 U/L in the sham group, 3,423 ± 410.9 UL in the IR group, and 2,928.57 ± 755.32 U/L in the IL-18BP group. There was a statistically significant difference between the IR group and sham group ($P = 0.034$). The blood and statistical analysis results of the groups are summarized in Table 1.

Assessment of blood cytokine levels

The blood level of IL-6 was 99.92 ± 2.32 pg/mL in the sham group, 107.86 ± 4.76 pg/mL in the IR group, and 100.29 ± 6.1 pg/mL in the IR + IL-18BP group. There was a statistically significant reduction in IL-6 levels between the IL-18BP treatment group and the IR group ($P = 0.008$). The blood level of IL-1β was found to be 39.93 ± 4.24 pg/mL in the sham group, 47.72 ± 7.63 pg/mL in the IR group, and 43.65 ± 3.90 pg/mL in the IR + IL-18BP treatment group. The data among the groups were close to significance in the statistical analyses ($P = 0.053$). The blood level of IL-18 was 170.73 ± 6.82 pg/mL in the sham group, 190.3 ± 14.3 pg/mL in the IR group, and 175.87 ± 8.19 pg/mL in the IR + IL-18BP group. There was a statistically significant reduction in IL-18 levels between the IL-18BP treatment group and the IR group ($P = 0.006$). The blood level of TNF-α was 130.94 ± 6.57 pg/mL in the sham group, 142.74 ± 6.92 pg/mL in the IR group, and 133.67 ± 5.33 pg/mL in the IR + IL-18BP group. Although reduced levels were achieved in TNF-α with the treatment, the finding was significance in the statistical analyses among the groups ($P = 0.007$). The blood level of IFN-γ was 58.66 ± 5.23 pg/mL in the sham group, 78.61 ± 8.35 pg/mL in the IR group and 64.98 ± 6.58 pg/mL in the IR + IL-18BP group. The reduction in the IFN-γ level with the treatment in IR + IL-18BP group was statistically significant ($P < 0.001$). The relations among the groups regarding the blood levels of IL-1β, IL-6, IL-18, TNF-α, and IFN-γ are summarized in Table 1.

Assessment of TAC, TOC, and OSI levels

The TOC data were considerably higher (7.85 [7.08–8.42]) in the IR group compared to the other groups; however, it was significantly reduced (4.95 [4.45–5.92]) in the IR + IL-18BP group. The TAC results were similar among the groups; however, the OSI value was statistically significantly reduced in IR + IL-18BP group (439.03 ± 165.5) compared to the IR group (695.28 ± 139.02) ($P = 0.008$). The TOC, TAC, and OSI data, and the statistical analysis results are summarized in Table 1.

Histopathological and immunohistochemical examination findings

Morphological change and H-Score results were given in Table 2 and Fig. 1. There was a prominent difference between the IR group and the other two groups by means of inflammatory cell infiltration ($P < 0.05$). Also, there was significant difference by means of sinusoidal enlargement and necrotic cell density mass between the IR applied only group and controls and between the IR and IR + IL18BP groups ($P < 0.05$) (Table 2, Fig. 1). In immunohistochemical evaluation, there was a prominent difference between the IR and sham groups ($P = 0.001$); between IR and IR + IL18BP groups ($P = 0.026$) by means of iNOS expression levels (Mean H-Score) (Table 2, Fig. 1).

DISCUSSION

To the best of our knowledge, the protective effect of IL18-BP in hepatic IR injury following infrarenal aortic occlusion model of rats has not been studied. In the present study, we showed that hepatic IR injury, which can be developed during aortic surgery, was reduced partially by IL18-BP. IL18-BP ameliorated the disorders of hepatic functions and decreased serum levels of inflammatory cytokines like TNF-α, IL-6, and IL-18 related to infra-aortic IR injury. At the same time, IL18-BP reduced the serum levels of OSI in the liver of infra-aortic occlusive rats and also ameliorated histopathological disorders induced by IR injury compared to sham group. The hepatic IR injury, which is a complex procedure involving intracellular pathophysiological signal pathways, cytokines, and mediators, is based on free oxygen radical (FOR) derivatives that occur due to the molecular oxygen inflow to the cell during the period of reperfusion, where the blood supply is restored upon the depletion of energy storages and deposition of toxic metabolites as a result of insufficient perfusion of the cells during ischemia [10]. As a result of the deposition of neutrophils and activation of xanthine oxidase in the endothelial cells secondary to the inflammatory incident, FORs such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO–), and lipid peroxidation occur. Lipid peroxidation is held primarily responsible for the disintegration of the hepatic cell membrane [11]. The hepatic damage exacerbated due to the reperfusion results in the destruction of lipids, essential cellular proteins, and DNA. Cellular damage, DNA fragmentation, apoptosis, and increased activity of nuclear kappa B (NF-κB) cause local

Table 2. Morphological evaluation and immunohistochemical scoring of the groups

Group	Inflammatory cell migration	Edema	Sinusoidal enlargement	Necrotic cell density	Total	iNOS expression level
SHAM	0	1	0	0	1	10
IR	2*	1	3*	4*	10*	72.5*
IR + IL-18BP	0†	1	1†	1†	3†	51.8†

IR, ischemia/reperfusion; IL-18BP, IL-18 binding protein; iNOS, inducible nitric oxide synthase.
*P < 0.05 *vs.* Sham. †P < 0.05 *vs.* IR.

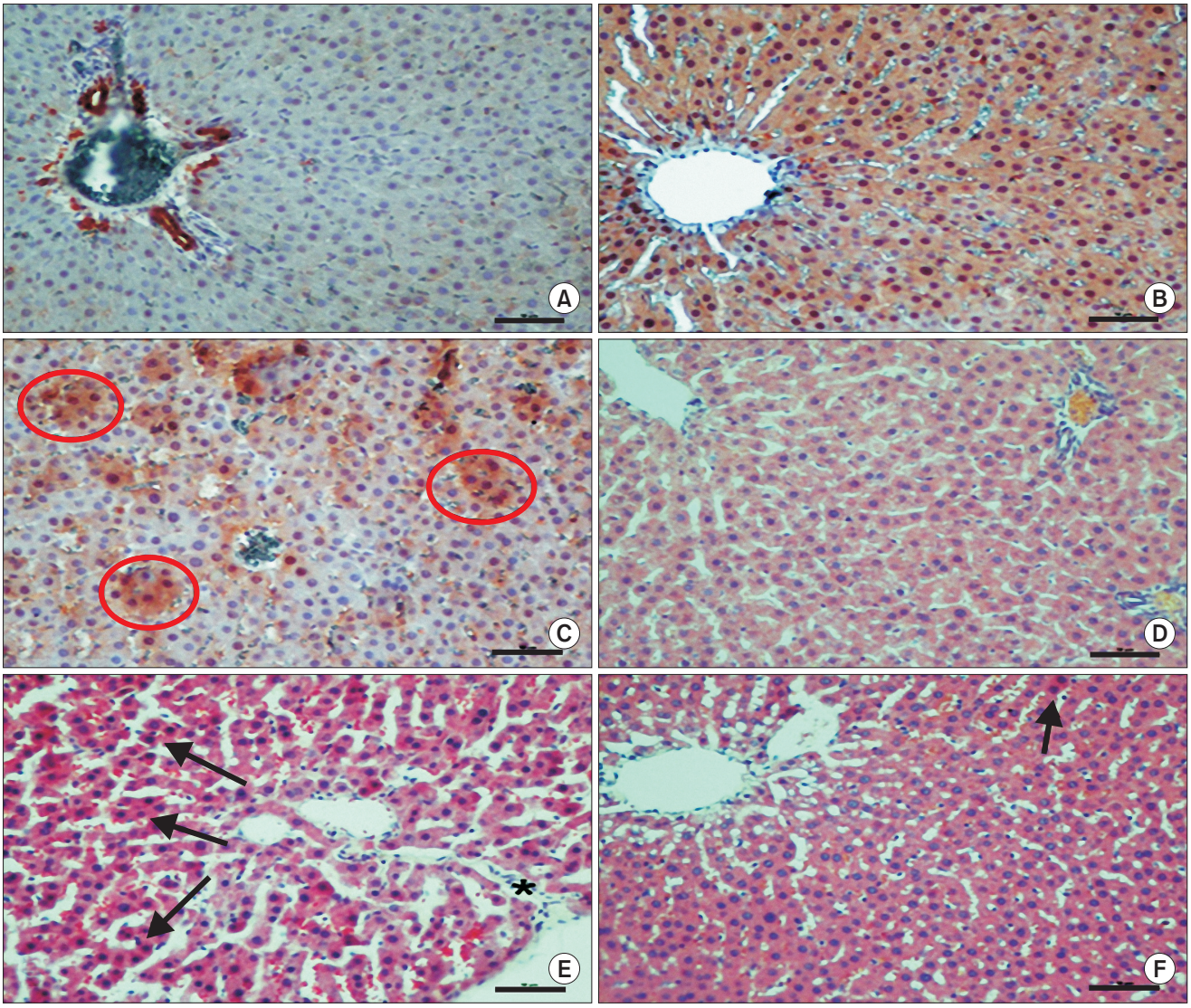

Fig. 1. (A) Inducible nitric oxide synthase (iNOS) staining for sham group. A few slight and mild immunopositive cells are seen around central veins which is normal because liver has massive metabolism, and oxygen saturation is considerably low especially around central veins (iNOS primary antibody, ×40). (B) iNOS staining for ischemia/reperfusion (IR) group. It is obviously seen that virtually whole hepatocyte cells were stained with iNOS from mild to heavy degrees (iNOS primary antibody, ×40). (C) Liver tissue taken from IR + IL-18 binding protein (IL-18BP) group stained with iNOS. Immunopositivity and number of the stained hepatocyteds are decreased and a few immunopositive cell clusters are seen in paranchyme (circles) (iNOS primary antibody, ×40). (D) General view of the liver tissue of sham group. Very slight edema is seen in paranchyme. But there is no inflammatory cell migration and sinusoidal enlargement (H&E, ×40). (E) Liver tissue taken from IR group. Slight edema, moderate sinusoidal enlargements, diffused necrotic cell groups (arrows) and mononuclear cell infiltration (asterix) are seen in paranchyme (H&E, ×40). (F) General view of the liver tissue of IR/IL-18 BP group. Very slight edema and slight sinusoidal enlargements are seen in paranchyme. But there is no inflammatory cell migration and there are a few necrotic cells (arrow) (H&E, ×40).

and systemic organ damage [12]. Proinflammatory cytokines such as IL-1, IL-6, IL-18, TNF-α, and IFN-γ have important roles, especially in the pathophysiology of reperfusion injury [13]. Hepatic IR injury is a complex mechanism involving many cytokines, endothelin, and oxygen free radicals and it may also frequently appear in the clinical practice after liver trauma, liver transplantation, and liver resection. Hepatic IR injury, rather than being an inflammatory response that affects not only the liver, is a systemic inflammatory chain affecting other distant organs, especially the kidneys and the lungs [14]. Many medications and chemical components have been studied in hepatic IR injury at an experimental level. However, there are only a few used in routine practice due to the side effects. IL-18, which is a member of the IL-1 cytokine family, is also called IFN-γ inducing factor. IL-18, a proinflammatory cytokine, is synthesized by multiple immune cells. IL-18 thus requires processing by caspase 1 to become an active cytokine. Moreover, IL-18 has been found to be significantly upregulated in both serum and inflammatory tissue [15]. It is known that the damage that occurs due to IL-18 is independent from the neutrophils and CD-4 T cells. IL-18 mainly modulates the activity of macrophages and thereby causes the production of IL-1B, IL-6, macrophage inflammatory protein (MIP)-1, MIP-2 and monocyte chemotactic protein-1 [16]. It is also known that IL-18 has a role in the production of IFN-γ from the TH1 and NK cells in an inflammatory setting. The Kupffer cells, macrophages, and antigen-presenting cells are responsible for the production of IL-18 in the liver. Particularly during the period of reperfusion, the hepatic Kupffer cells are activated in a short time [17]. It is a known fact that the main producer of IL-18 is Kupffer cells in early hepatic IR injury. IL-18BP is a natural inhibitor of IL-18 and it binds to the IL-18 cell surface receptors rather than IL-18. Therefore, the balance between IL-18 and IL-18BP has an important role in the inflammatory setting. IL-18BP was first identified in the urine of healthy individuals and purified by using IL-18-coupled beads [18]. IL-18BP is excreted following glomerular filtration. IL-18BP is thought to be a soluble decoy receptor because it can specifically block the binding of mature IL-18 to its authentic receptor and thereby inhibit IL-18-induced IFN-γ production [19]. Although there are a limited number of studies related to IL-18BP in the literature, it was demonstrated that IL-18BP had a free-radical scavenging effect due to its suppressive feature on the levels of TNF-α, IL-1β, and IL-6 in various inflammatory IR models. A study by Venkatachalam et al. [20] established that IL-18 neutralization had a protective effect on myocardial ischemia and reperfusion injury in the myocardial IR injury that occurs secondary to the ligation of the anterior descending coronary artery. In another study by Wang et al. [21], they observed a reduction in the IL-18 levels and apparent recovery in the acute lung injury to be secondary to this reduction upon the administration of breviscapine in the acute lung IR injury. IL-18 and the neutralization of IL-18 through pharmacological agents were mostly studied in IR injury. The study by Wang et al. [22] clearly established that IL-18 neutralization prevented apoptosis in the renal epithelial cells and increased epithelial proliferation, inhibited the infiltration of macrophages, reduced the levels of inflammatory cytokines, and increased the expression of vascular endothelial growth factor; however it reduced the level of thrombospondin-1. The studies by Wu et al. [23] and Leslie and Meldrum [24] explicitly demonstrated the protective effect of IL-18 neutralization in renal IR injury. The elevated IL-18 levels in the distant organ lung injury that occur secondary to ischemia and reperfusion are at the center of primary pathophysiological reactions along with TNF-α/iNOS activity. The study by Yang et al. [25] clearly demonstrated the primary role of IL-18 in the distant organ lung inflammatory injury that occurs secondary to intestinal IR injury. Additionally, the role of IL-18 in lung injury that occurs secondary to hepatic IR injury was evidently observed in a study by Takeuchi et al. [26]. Takeuchi et al. [26] also established the protective effect of IL-18 neutralization with antibodies in the proinflammatory cytokines, NF-κB, and activator protein-1, and hepatic ischemia and reperfusion injury secondary to apoptosis. Furthermore, the rat model study by Li et al. [27] clearly demonstrated that IL-18BP inhibited the infiltration of macrophages. In the present study, the blood levels of AST, ALT, and LDH were considerably higher in the IR group compared to the sham group. This elevated biochemical parameter, which was also supported histopathologically, demonstrated that the presence of hepatic IR injury occurred after the infrarenal aortic clamping. Nevertheless, the levels were found statistically significantly reduced in the IR + IL-18BP treatment group after the treatment. With regards to the blood levels of IL-1β, IL-6, IL-18, TNF-α, and IFN-γ, the levels of all inflammatory cytokines except IL-1β were statistically significantly reduced upon the administration of exogenous IL-18BP compared to the IR group.

The increased inflammatory response in IR injuries leads to an increase in TOS. TOS varies based on the balance between the oxidative and antioxidant substances. The balance usually acts unfavorably for the antioxidant substances in the inflammatory incidents. Although it is possible to measure the level of OS by solely identifying the oxidant or the antioxidant components within the system, determining both the oxidant or antioxidant components is a more effective approach [28]. Thus, to be able to determine OS more accurately, both the level of oxidants and antioxidants were assessed. The current study measured the OSI, which reflects the ratio between the oxidants and antioxidants. The TAC and the TOS of the system were measured as well. Based on the study results, the authors observed that IL 18-BP administration led to a significant decrease in OSI and TOS levels in liver serum, and

to a significant increase in TAC levels. As OS is known to trigger the generation of inflammatory cytokines and cell adhesion molecules, it is possible that the inflammatory reaction caused by endotoxemia following hepatic ischemic reperfusion might be associated with OS. In the present study, an increase in the TAS levels and a reduction in TOS and OSI levels were achieved in the IL-18BP treatment group. The authors believe that this setting may have resulted from the natural antioxidant characteristics of IL-18BP. Additionally, IL18-BP treatment alleviated pathological structural changes.

Previous studies have indicated a correlation between nitric oxide and interleukin synthesis. The study by Kim et al. [29] reported that lipopolysaccharides triggered higher plasma IL-1 levels in animals that were iNOS-deficient. Another study demonstrated that IL-6 can mitigate the damages associated with ischemic reperfusion injury in rodents [30]. The authors of the current study observed that interleukin 18BP administration decreases the intensity of iNOS signaling in serum subjected to hepatic ischemic reperfusion. This observation is in agreement with the findings of previous studies.

An important limitation of the current study was associated with the fact that although IL-18BP preserves its activity in different species, the structural similarity between human and rat IL-18BP is limited. For this reason, it would be preferable to reassess the protective effects afforded by IL-18BP against hepatic ischemic reperfusion damage in rat models by specifically using murine IL-18BP.

In conclusion, the current study results demonstrated that IL-18BP administration reduced the observed levels of inflammatory cytokines (e.g., TNF-α, IL-6, and IL-18) following hepatic ischemic reperfusion injury, while also mitigating the impairment of liver functions. In addition, it was also observed that IL-18BP decreased OSI levels observed in the serum of rats subjected to hepatic ischemic reperfusion injury. The authors believe that this study data will contribute to the existing body of knowledge regarding IL-18BP's role and involvement in immunoregulation. The current study results are related to hepatic ischemia reperfusion injury, and are hence relevant with regards to injury that occurs during liver surgeries (e.g., hepatectomy). Increasing the level of IL-18BP expression can potentially reduce liver damage during surgery by enhancing inherent protective mechanisms. However, prior to any clinical trials with IL-18BP, it is important to conduct further studies on various animal models in order to better ascertain the mechanisms of cytokine's protective effects. In this context, this is the first study evaluating the favorable effect of melatonin on IR-exposed liver injury after infrarenal occlusion of the aorta with our ischemic reperfusion (30–120 minutes) period. We supposed that our results will put forward a new point of view to the literature about protective, antioxidant and anti-inflammatory effect of IL-18BP on liver functions of IR after infra-renal aortic occlusions.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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