# Antioxidant, Antiapoptotic and Inflammatory Effects of Interleukin-18 Binding Protein on Kidney Damage Induced by Hepatic Ischemia Reperfusion

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## ABSTRACT

**Objective:** Acute kidney injury (AKI) is a serious condition that can be induced by liver transplantation, major hepatic resection or prolonged portal vein occlusion. The AKI can increase the frequency of postoperative complications. In the current study, we aimed to investigate whether interleukin-18 binding protein (IL-18BP) pretreatment has a protective effect against possible kidney injury–mediated liver ischemia-reperfusion (IR) achieved by Pringle maneuver in an experimental rat model.

**Materials and Methods:** A total of 21 Wistar albino rats were included in this study. Animals were equally and randomly separated into 3 groups as follows: Sham ($n = 7$), IR group ($n = 7$) and IR + IL-18BP group ($n = 7$). Serum aspartate transaminase, alanine aminotransaminase and lactate dehydrogenase enzyme activities and serum urea and creatinine levels were determined. Tumor necrosis factor-α, IL-6, IL-1β, interferon gamma, total oxidant status, total antioxidant status and oxidative stress index were measured in kidney tissue homogenate samples. Histopathological examination and immunohistochemical Caspase-3 staining were applied to examine the general morphologic structure and apoptosis.

**Results:** Renal total oxidant status; oxidative stress index; IL-18 levels; serum aspartate transaminase, alanine aminotransaminase and lactate dehydrogenase activities and creatinine levels were significantly lower in IR + IL-18BP group, when compared with the IR group. Beside this, total antioxidant status levels were remarkably higher in IR + IL-18BP group, when compared with the IR group. The caspase-3 expression degree in IR group was remarkably higher than other groups.

**Conclusions:** It has been demonstrated that IL-18BP pretreatment may have inflammatory, antioxidant and antiapoptotic effects against AKI induced by hepatic IR.

**Key Indexing Terms:** IL-18BP; Protective effect; Liver ischemia-reperfusion injury; Remote organ; Kidney injury. **[Am J Med Sci 2016;351(6):607–615.]**

## INTRODUCTION

Ischemia-reperfusion (IR)–induced liver damage is a pathologic condition in which oxidative stress and reactive oxygen species (ROS) play important role in tissue destruction. Under some conditions such as major hepatic surgery, resection or liver transplantation in which temporary occlusion of the portal vein is mandatory, blood flow is ceased for a relatively long period. Hepatic blood flow may also be depleted in some cases including hemorrhagic shock, several trauma, late-stage sepsis, hepatic artery ligation, vascular lesions and liver injuries.[1,2] Deleterious effect of IR is more than that of the ischemia process alone. The ROS and toxins which are produced during IR process can affect some other organs including lungs, kidneys and heart. Continuous or intermittent vascular clamping of the hepatoduodenal ligament procedure is also known as Pringle maneuver. This maneuver method has become a routinely used method to control hepatic hemorrhages. However, it may also cause liver ischemia attributed to decrease in blood flow, venous congestions in spleen, hemodynamic instability and damage in lungs, kidneys and other remote organs.[3,4] The IR-induced tissue damage occurs during the perfusion process and ROS have been implied in this condition.[5] Acute renal failure may be associated with acute liver ischemia in 40-85% of the cases depending on diagnosis and other associated factors.[6] Especially in cases of large hepatic resection, liver transplantation and septic conditions, consequent acute renal failure can seriously increase the mortality rates. However, the pathophysiological mechanisms underlying IR-induced renal failure have not been fully revealed yet and there is still ongoing research in this field to find a suitable treatment modality.[7]

Interleukin-18 (IL-18) is a proinflammatory cytokine produced by macrophages, epithelial cells and active T lymphocytes, which contribute to inflammatory reactions such as regulation of macrophage activity along with tumor necrosis factor (TNF) and IL-1 production in mononuclear cells. The IL-18 also plays an important role in immune response and inflammation. Various normal or malignant cells can give rise to cellular response via IL-18 receptors (IL-18R).[8,9] Caspase-3 activation has been closely linked with inflammation via processing of IL-1β and IL-18.

IL-18 binding protein (IL-18BP) is a natural inhibitor of IL-18, which has affinity to IL-18 more than the cell membrane surface receptors.[10] The equilibrium between IL-18 and IL-18BP is an important factor that may affect the severity of some inflammatory disorders. The IL-18BP has been used in rheumatoid arthritis treatment as an inflammatory agent and it has also been used as a therapeutic agent in some studies.[8] It has been reported that IL-18BP-Fc compound exhibited protective effect against Fas/FasL-mediated hepatic damage via inhibiting IL-18.[11] In other experimental studies conducted on acute renal ischemia models, it has been indicated that IL-18BP has a protective effect that is attributed to its inflammatory effect.[11,12]

Although it has been indicated that IL-18BP decreased IR damage in different experimental animal models, there is no such study conducted to find out the possible effects of IL-18BP on renal damage in hepatic IR model in the literature so far. In this study, we aimed to investigate the possible inflammatory, antioxidant and antiapoptotic effects of IL-18BP on kidney remote organ damage due to hepatic IR injury via analyzing liver function tests (serum aspartate transaminase [AST], alanine aminotransaminase [ALT] and lactate dehydrogenase [LDH] activities), kidney function tests (serum urea and creatinine levels), oxidative stress parameters (kidney total antioxidant status [TAS], total oxidant status [TOS] and oxidative stress index [OSI] levels), proinflammatory cytokines (kidney TNF-α, IL-6 and IL-1β levels), liver and kidney histopathological examinations and caspase-3 expression by immunohistochemical staining in kidney tissue samples.

## MATERIALS AND METHODS

### Experiment Animals

A total of 21 Wistar albino rats weighing between 200 and 250 g were included in this study. Study protocols and experimental methods were approved by the local Institutional Ethics Committee of Experimental Animals of Afyon Kocatepe University. Rats were kept under standardized animal laboratory conditions that comply with the "Experimental Animal Usage and Principles regulated by National Health and Medical Research Council" and "Guide for Experimental Animal Care and Usage prepared and issued by National Institution of Health" (NIH issue no. 85–23, 1985 revised). All animals were given enough feed *ad libitum* and starved overnight before the experimental procedure.

### Study Design

#### *Study Groups*

Animals were equally and randomly separated into 3 groups as discussed below:

*Sham Group (Sham, n = 7).* Only abdominal incision was performed and closed after 60 minutes. No additional treatment was given.

*IR Group (IR, n = 7).* Saline solution (0.9%, 1 mL) was intraperitoneally administered 30 minutes before the surgical intervention. Consequent hepatoduodenal ligament dissection and Pringle maneuver were performed for 60 minutes. Clamps were removed after 60 minutes of ischemia process and livers were reperfused for 2 hours. No additional treatment was given to this group.

*IR + IL-18BP Group (IR + IL-18BP, n = 7).* IL-18BP (100 μmg/kg, b.wt.[13] reconstituting at 100 μg/mL in sterile phosphate-buffered saline) was intraperitoneally injected 30 minutes before the Pringle maneuver. Clamps were removed after 60 minutes of ischemia process and livers were reperfused for 2 hours. Human and murine IL-18BP isoforms are active across species.[14] Therefore, we preffered to use the recombinant human IL-18BP (119-BP; R&D Systems, Minneapolis, MN) in the current study.

### Surgical Procedure

Rats were anesthetized with intramuscular ketamine (40 mg/kg) (Ketalar, Parke-Davis, Eczacibasi, Turkey) and subsequent 5 mg/kg Xylazine (Rompun, Bayer, Turkey) intramuscular injections for premedication. Whole animals were then fixed on tables to avoid uncontrolled movements and covered with sterile dressings. Abdominal median laparotomy was performed under sterile conditions and ischemia was induced by Pringle maneuver–clamping portal triad with atraumatic microvascular bulldog clamp (Vascu-Statt, Mini straight 1001-502; 800-328-9458/USA and Canada) and lasted for 60 minutes. Abdominal incision was temporarily covered up by a plastic clothing to minimize the heat loss and dehydration. Animals were kept under lamps for 60 minutes for temperature control and vital signs were followed until the anesthesia effect went down. After this time, the ischemic liver was reperfused and this period was continued for 120 minutes. At the end of the procedure, 3 mL of blood samples were collected from the abdominal aortas for biochemical examinations. Afterwards, rats were euthanized by exsanguination through the abdominal aorta.

Liver and kidney tissue samples were collected for biochemical, histopathological and immunohistochemical