The effect of bisoprolol on serum levels of liver enzymes AST, ALT and liver tissue in hepatic ischemia-reperfusion model in male rats.

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Abstract

Background and Aim: Hepatic ischemia-reperfusion (IR) injury is a systemic process that involves different organs and originates usually after hepatic surgeries and transplantations. In some studies, beta-blockers such as bisoprolol (BSP) have shown promising results in reducing the IR-caused injuries to the liver. The present study investigates the effects of BSP administration on serum levels of hepatic enzymes AST and ALT and hepatic tissue in male rats after hepatic IR injury.

Materials and Methods: A total of 28 Wistar rats were randomly split into four groups (each with 7 rats), including 1) Sham (receiving 2 ml/day normal saline by oral gavage for 7 consecutive days, followed by surgery without IR induction), 2) Sham + BSP (receiving 10 mg/kg BSP by oral gavage for 7 consecutive days, followed by surgery without IR induction), 3) IR (receiving 2 ml/day normal saline by oral gavage for 7 consecutive days, followed by surgery with IR induction), and 4) IR + BSP (receiving 10 mg/kg BSP by oral gavage for 7 consecutive days, followed by surgery with IR induction). After taking blood samples from the heart, rats were euthanized by surgical incisions on the diaphragm. Then, hepatic resection was performed for further histopathological investigations.

Results: The mean serum level of AST was insignificantly grown in the IR group compared to the Sham group ($p>0.05$), while that in the IR + BSP group was insignificantly reduced compared to the IR group ($p>0.05$). Similarly, the mean serum level of ALT in the IR group was insignificantly increased compared to the Sham group. And ultimately, the mean serum level of ALT was reduced slightly in the IR + BSP group, but this decrease was not statistically significant compared to the IR group ($p>0.05$). Furthermore, the structure of hepatic cells was found to be normal in all groups, and the cells did not alter structurally after BSP administration.

Conclusion: The results of this study showed BSP pretreatment before hepatic IR probably cannot have a significant effect on liver tissue and some liver enzymes including AST and ALT.

Keywords: Hepatic ischemia-reperfusion injury, Hepatic enzymes, AST, ALT, Bisoprolol, Rat.

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Introduction

Hepatic ischemia-reperfusion (IR) injury is a systemic injury and the leading cause of hepatic dysfunction after hepatic surgery or transplantation, involving different organs and tissues. IR may produce irreversible damage and lead to multiple organ dysfunction syndrome (MODS) and even death [1]. Thus, the issue of IR prevention is a subject worth exploring. The most potent contributors to IR include oxidative stress, inflammatory processes, and damage to cellular mitochondrial functioning [2, 3]. Some injuries arise from ischemia and become worsened upon the onset of reperfusion due to the production of oxidants that trigger oxidative and inflammatory processes. Current therapies against IR perform by interfering with the function of these harmful factors.

Zhang et al. (2017) investigated the cardioprotective effects of bisoprolol (BSP) against myocardial IR. The results revealed that BSP confers cardioprotective effects by enhancing postischemic cardiac function, decreasing infarct size, reducing apoptotic index, diminishing serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH), suppressing the secretion of inflammatory factors (e.g., TNF-α and IL-6), and reducing the response of unfolded (defective) proteins. BSP was further found to prevent IR-caused damage by conferring antioxidative and anti-inflammatory effects [4].

Wang et al. (2020) investigated the protective role of BSP in cardiomyocytes against IR in male rats. After IR induction, rats in the intervention group were prepared with BSP by oral administration before 0.5 h ischemia/4 h reperfusion. BSP reduced infarct size in the BSP-treated group ($p<0.05$) and decreased the level of inflammatory and oxidant factors in treated rats [5].

Nishio et al. (2008) assessed the therapeutic effects of BSP on the survival of hypertensive diastolic heart failure model rats. In their study, left ventricular hypertrophy and the expression of proinflammatory factors were significantly attenuated in the treated group compared to control rats [6].

In a 2001 randomized clinical trial, Poldermans et al. screened 1351 patients before surgery and found that BSP can markedly reduce long-term cardiac death and myocardial infarction after successful major cardiac vascular surgery [7].

In a 2017 empIRcal study, Balzan et al. investigated the effect of pretreatment metoprolol injection in ameliorating hepatic IR in pigs and found that metoprolol does not diminish the level of hepatic enzymes such as AST (aspartate aminotransferase), ALT (alanine aminotransferase), and ALP (alkaline phosphatase). However, their study was contested because of using hepatic enzymes that

are not specific to IR, not measuring markers that are specific to cell and tissue damage, and not reflecting the oxidant and anti-oxidant profiles in the participants [8].

Hassan et al. (2021) evaluated the impact of carvedilol (CAR) against hepatic IR in male rats and found a significant increase in the post-IR level of hepatic enzymes such as AST, ALT, and ALP, which serve as a marker of hepatic damage. CAR treatment before hepatic I/R reduced the intensity of hepatic damage and resulted in the restoration of hepatic enzymes compared to the control group. Advantageously, their study identified specific markers of cell and tissue damage and the oxidant and antioxidant profiles of the rats. They ultimately reported an improvement in the level of these hepatic markers [9].

Castagno et al. (2010) studied the effect of BSP in improving heart failure (HF) in patients with heart HF and concomitant renal impairment. They found that the use of BSP in patients with heart failure and concomitant renal impairment can confer beneficial results [10].

The level of AST is increased when the tissues and cells containing this enzyme are damaged. An increase in AST levels can occur six hours post tissue damage. Normal levels of AST are typically high from birth to 3 years, compared to AST levels in children and adults. An AST blood test (previously called SGOT) measures the amount of aspartate transferase in the blood released from damaged tissues. Similarly, An ALT test (also called SGPT) measures the level of alanine aminotransferase in the blood. ALT is an enzyme found mostly in (and often produced by) hepatic cells [11].

BSP is a beta-blocker (i.e., a selective beta 1 receptor blocker). Recently, Wang et al. [5] investigated the impact of BSP on cardiac IR in rats. In their study, male Sprague Dawley (SD) rats were prepared with BSP by oral administration before 0.5 h ischemia/4 h reperfusion. They revealed a significant impact of BSP on diminishing the rate of cell destruction and found that the level of cardiac enzymes in the BSP-treated is increased compared to the control group. Based on the cellular assessments of heart tissue, BSP was found to exert its protective impact against IR via the PI3K/AKT/ GSK3β pathway. Similar studies have also shown that BSP can exert its effect by producing and releasing nitric oxide (NO). Thus, BSP can be effective in heart damage, as the produced NO can reduce hepatocellular apoptosis, oxidative stress, and leukocyte attachment, thus improving mitochondrial functioning [4, 5].

Based on the literature, beta-blockers such as BSP can control oxidative stress and enhance mitochondrial functioning. It further can produce and release NO. And since NO plays a key role in reducing hepatocellular apoptosis, oxidative stress, and leukocyte attachment, thus improving mitochondrial functioning, this class of drugs (particularly BSP) can be effective in the improvement of hepatic damage [4, 5]. Taking note of the many hepatic surgeries and transplantations carried out worldwide and the protective impact of beta blockers against IR, the present study aims to investigate the effect of BSP on the level of some hepatic enzymes and the structure of hepatic tissue.

Materials and Methods

The present experimental study was carried out in the Physiology Laboratory of Yasuj University of Medical Sciences (YUMS, Yasuj, Iran). A total of 28 white male Wistar rats (weighted 180 to 250 gr; allocated to four 7-member groups) were randomly selected from the Center to Maintain and Breed Laboratory Animals at YUMS. Rats were kept at a standard temperature of 22±2°C and under the normal conditions of a 12:12-h light-dark cycle, with free access to standard food and water. While having free access to water, rats were starved for food for 16 to 18 hours before the experiments. They were anesthetized by ketamine and xylazine (75+7.5 mg/kg/ip) [12].

After shaving and then sterilizing the abdomen with saline and betadine, the rats underwent laparotomy surgery. For this, an incision (approximately 3-4 cm) was made in the abdominal mid-line to allow for the careful removal of intestines and holding them far away from the abdominal cavity using cotton swabs until the portal vein (PV) and its structures appear. Then, the hepatic quadrate lobe was carefully separated from the left lobe to reveal the portal triad (containing extrahepatic segments of the PV, hepatic artery, and bile ducts). For IR induction, a small vessel clamp was carefully placed around the PV and hepatic artery narrowly above the bifurcation of the hepatic right lobe. During IR induction, the middle and left lobes (constituting nearly 70% of the liver) underwent instant decoloration, i.e., converting from the normal red-brownish color to pale brown. After clamping, the intestines were returned to the abdominal cavity [13]. The clamp was removed 30 minutes post IR induction to allow suturing of the abdominal muscles and then the abdominal skin. Reperfusion was then performed for 30 minutes [14].

Study groups

1. **Sham group:** Receiving 2 ml/day normal saline by oral gavage for 7 consecutive days, followed by surgery without IR induction)
2. **Sham + BSP group:** Receiving 10 mg/kg BSP by oral gavage for 7 consecutive days, followed by surgery without IR induction)
3. **IR group:** Receiving 2 ml/day normal saline by oral gavage for 7 consecutive days, followed by surgery with IR induction)
4. **IR + BSP group:** Receiving 10 mg/kg BSP by oral gavage for 7 consecutive days, followed by surgery with IR induction).

Preparation of serum and liver tissues

After IR induction, blood samples were taken from the heart and the rats were then euthanized by an incision in the diaphragm. A liver biopsy was performed to remove a small piece of liver tissue for quantifying the intensity of tissue damage. The tissues were fixed in formalin. Blood samples

were kept in tubes to clot. After clotting, the samples were centrifuged for 5 min at 5000 rpm at 37°C, and the supernatant was kept to further measure the study variables [14, 15].

Liver tissue samples were stored in special containers containing formalin (10%). Once the tissues were fixed by a tissue processor and embedded in paraffin, they were cut into sections by a microtome to allow placing them on slides. The prepared slides were stained with hematoxylin-eosin (H&E) and examined by a light microscope.

Data were statistically analyzed in SPSS software using one-way ANOVA and post hoc tests (LSD or Dannet) to compare the mean values between groups. The results with $p<0.05$ were pondered to be statistically significant.

Findings

As demonstrated in Figure 1, the mean serum level of AST increased in the IR group compared to the sham group but this increase was not significant ($p>0.05$). The serum level of AST in the "IR+BSP" group decreased compared to the IR group, but this decrease was not significant ($p>0.05$). These results indicate that administration of BSP at this dose probably cannot significantly reduce the serum level of liver enzyme AST in rats after hepatic IR injury.

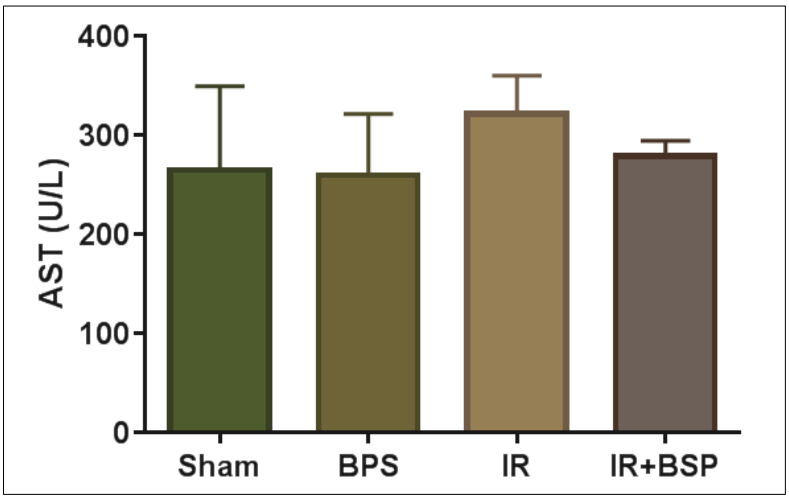

Figure 1. The post-IR effects of BSP pretreatment (2 ml/day normal saline by oral gavage for 7 consecutive days) on the serum levels of AST. Data are given based on mean ± SEM (n: 7). IR: ischemia-reperfusion group; IR + BPS: ischemia-reperfusion receiving BSP; AST: aspartate aminotransferase; IR: ischemia-reperfusion; BSP: bisoprolol; N/S: normal saline

As shown in Figure 2, the serum level of ALT in the IR group was significantly increased compared to the sham group ($p<0.01$). and treatment with bisoprolol decreased the level of ALT, although this decrease was not significant ($p>0.05$). these results indicate that bisoprolol probably with this dose, it cannot significantly decrease the serum level of ALT in the ischemia-reperfusion model in rats.

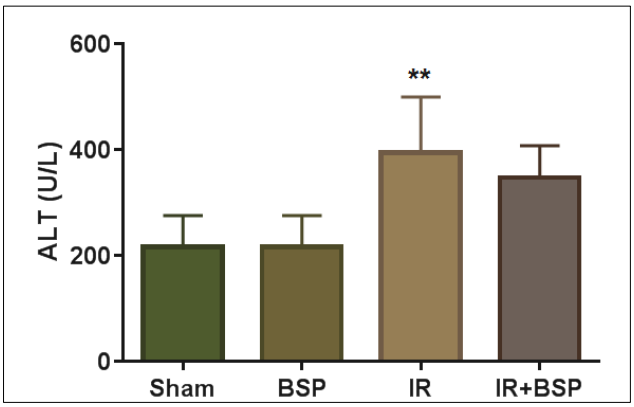

Figure 2. The post-IR effects of BSP pretreatment (2 ml/day normal saline by oral gavage for 7 consecutive days) on the serum levels of ALT. Data are given based on mean ± SEM (n: 7); ** $p<0.01$ indicates a significant difference with the Sham group. IR: ischemia-reperfusion group; IR + BPS: ischemia-reperfusion receiving BSP; ALT: alanine aminotransaminase; IR: ischemia-reperfusion; BSP: bisoprolol; N/S: normal saline

The Liver tissues after surgery were fixed in formalin and then sectioned and examined under a microscope after staining with H&E. The following images show the results in the Sham group (insets A1 and A2), the Sham + BSP group (insets B1 and B2), the IR group (insets C1 and C2), and the IR + BSP group (insets D1 and D2) are given in Figures 3 and 4. The structure of liver cells after receiving bisoprolol in group IR + BSP did not change significantly compared to Sham + BSP group.

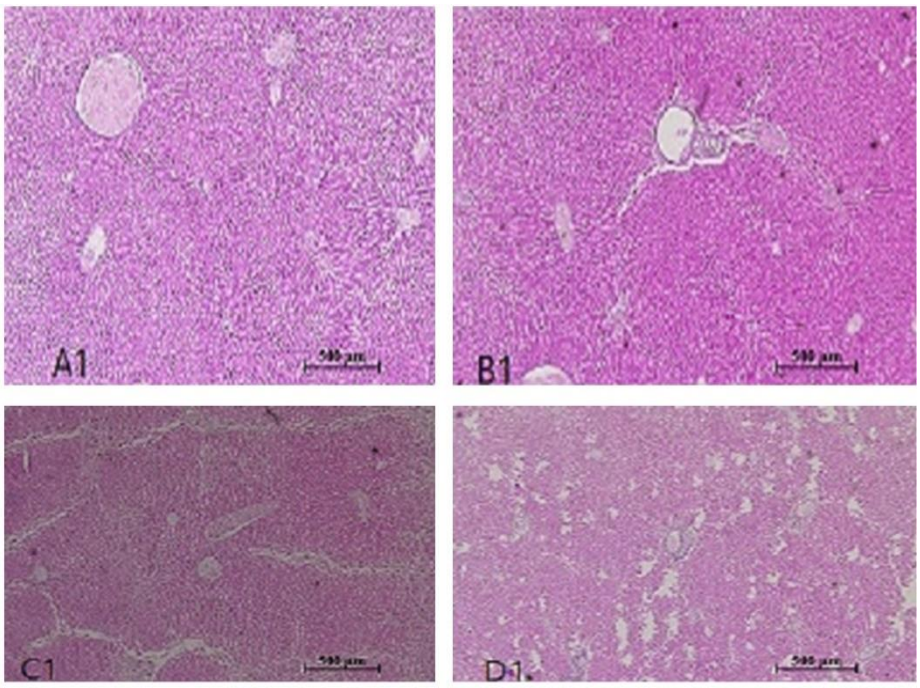

Figure 3. A1: Sham group; B1: Sham + BSP group; C1: IR group; and D1: IR + BSP group

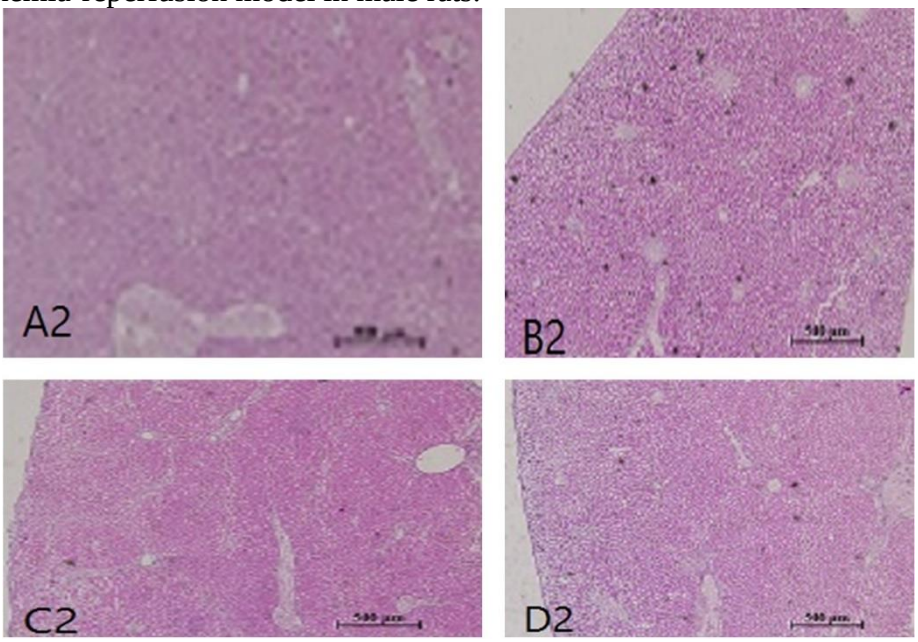

Figure 4. A2: Sham group; B2: Sham + BSP group; C2: IR group; and D2: IR + BSP group

Discussion

Hepatic IR injury is the leading cause of liver dysfunction post hepatic surgery or transplantation. In the present study, the serum level of AST in the IR group was higher than that in the Sham group, although this difference was not significant. In agreement with this finding, previous studies have also reported that hepatic IR injury can result in an elevated level of AST [9].

In the present study, the level of AST in the "Shap + BSP" group was narrowly decreased compared to the "Sham" group, though the difference in AST levels between the two groups was not significant. Additionally, AST levels in the IR+BSP group were insignificantly decreased compared to the IR group. Therefore, pretreatment with BSP probably cannot cause a significant decrease in serum AST level. This finding is contesting the results reported by other studies advocating that BSP pretreatment exerts antioxidant and anti-inflammatory effects and reduces hepatic injury and the level of liver enzymes such as AST. Contrary to the present study, Hassan et al. reported that carvedilol (a non-selective beta-blocker) significantly reduces AST levels in IR-treated pigs [9]. This finding is not consistent with the results of the present study. Such a difference can be due to using a different beta blocker, as Hassan et al. worked with a non-selective beta blocker (carvedilol) that can further affect the alpha receptor and exert a protective role via another mechanism, while the present study used BSP that is a selective beta-blocker.

Balzan et al. (2017) evaluated the effect of metoprolol in attenuating hepatic IR injury in pigs [8]. They found that metoprolol fails to significantly change the level of liver enzymes such as AST, compared to the control group. However, they did not investigate specific markers of cell damage and inflammation such as interleukin-6 (IL-6) and interleukin-10 (IL-10), antioxidant capacity,

and tissue necrosis factor. Similar to the present study, they further posited that their results could have been impacted by a low dose of the drug.

A reason for such heterogeneity in the results between the studies is employing different methodologies, such as differences in IR induction duration, various doses of medications, or concurrent muscle injuries during surgery. As mentioned earlier, AST is found in most tissues and is not liver-specific. Thus, its level may fluctuate as a result of other stimuli, such as surgery or muscle and heart injuries. These issues are thus recommended to be evaluated in future studies while using more specific markers for cell damage evaluation.

Research has revealed that the serum level of ALT is increased following hepatic IR. Similarly, the present study showed that the level of ALT in the IR group is significantly higher than that in the "Sham" group. Balzan et al. investigated the effect of metoprolol in ameliorating hepatic IR injury in pigs. They found that metoprolol fails to significantly alter the level of hepatic enzymes (e.g., ALT) compared to the control group. However, they did not investigate specific markers of cell damage and inflammation, antioxidant capacity, and tissue necrosis factor [8]. Similarly, the present study did not perform specific tests of cell damage and inflammation. The present study revealed that the serum levels of ALT in the "Sham + BSP" and "IR + BSP" groups were not significantly altered respectively compared to the "Sham" and "IR" groups.

In summary, ALT levels were increased following hepatic IR injury but did not significantly reduce after treatment with BSP. Although the effect of BSP on ALT levels was insignificant, the role of this medication in ameliorating hepatic injury cannot be overlooked and is still worth exploring with more specific markers of cell damage. Hassan et al. (2021) showed that carvedilol (a non-selective beta-blocker) can reduce tissue damage and ALT levels in rats exposed to hepatic IR [9]. However, this finding is not in line with our results, presumably due to using a different beta blocker, as Hassan et al. worked with a non-selective beta blocker (carvedilol) that can further affect the alpha receptor, while we used BSP that is a selective beta-blocker.

Conclusion

The present study revealed that BSP pretreatment before hepatic IR injury probably cannot significantly reduce liver enzymes ALT and AST. This finding agrees with the results of some other studies investigating the protective role of BSP and metoprolol, respectively in cardiac and hepatic IR injuries. For more clarification, future studies are recommended to assess specific markers of tissue cell damage and inflammation as well as oxidant and antioxidant factors.

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