Pretreatment Effect of Bisoprolol on the Serum Levels of ALP and LDH Enzymes in the Liver ischemia-reperfusion Model in Rats

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Abstract

Introduction: Liver ischemia-reperfusion injury occurs primarily after liver surgeries and liver transplants. In addition to the liver, it can involve systemically different organs of the body. In some cases, this injury is irreversible and may result in multi-organ dysfunction and death. Some studies report that bisoprolol can reduce the injury caused by ischemia-reperfusion in the heart and some other tissues. The present study investigates the effect of bisoprolol as a pretreatment on the serum levels of ALP and LDH enzymes in the liver ischemia-reperfusion model in male rats.

Methods: In this experimental study, 28 Wistar rats were randomly divided into 4 groups of seven (n = 7). They included the sham group that received 2 ml Normal Saline (NS) daily through gavage for 7 consecutive days and then surgery without induction of ischemia-reperfusion (IR), the BSP group that received 10 mg/kg BSP (dissolved in 2 ml of normal saline) through gavage for 7 consecutive days and then surgery without IR induction, IR group that received 2 ml NS daily through gavage for 7 consecutive days and then surgery and IR induction, and IR + BSP group that received 10 mg/kg BSP through gavage for 7 consecutive days, then surgery and IR induction. After the desired time, blood was collected from the heart of the rats. Then, the rats were euthanized by an incision in the diaphragm. After clotting and centrifugation of the blood samples, the upper part was removed to measure the desired factors.

Results: The mean serum concentration of ALP (alkaline phosphatase) in the BSP group did not change significantly compared to the Sham group ($P>0.05$). Also, the mean serum ALP level in the IR + BSP group did not change significantly compared to the IR group ($P>0.05$). The mean serum concentration of LDH (lactate dehydrogenase) in the IR + BSP group compared to the IR group and the BSP compared to the Sham group did not change significantly ($P>0.05$).

Conclusion: Based on the results, pretreatment with bisoprolol drug before the start of liver ischemia-reperfusion with the mentioned dose and the conditions of this research probably cannot cause a significant change in the serum levels of ALP and LDH enzymes.

Keywords: Liver, Ischemia-reperfusion, ALP, LDH, Bisoprolol, Rat

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Introduction

The liver plays a role in the detoxification of various metabolites and the production of many proteins and biochemical substances (1, 2). It also regulates glycogen storage, breakdowns red blood cells, and produces some hormones (3). It is primarily responsible for the metabolism of proteins, i.e. their synthesis and breakdown (4). It is responsible for the breakdown of many hormones also for breaking down and eliminating many waste products. liver plays a major role in the breakdown and neutralization of toxic substances and the metabolism of drugs (5).

Liver ischemia-reperfusion injury occurs primarily after liver transplantation and liver surgeries. This injury can systematically involve different organs of the body. It is sometimes irreversible and may result in multiple organ dysfunction and death. Owing to the high number of liver surgeries, this complication can affect a large number of people. Thus, conducting studies in this field is crucial. Some studies have examined the effect of beta blockers on ischemia-reperfusion injury (6, 7). The results of some studies have shown that these drugs can reduce the rate of injuries by reducing lipid peroxidation, reducing inflammatory cytokines, and preventing oxidative stress.

One of these experimental studies has examined the effect of beta-blocker metoprolol (beta blocker) on the injury caused by ischemia-reperfusion of the pig heart. It was concluded that this drug has no significant effect on the injury caused by ischemia-reperfusion. However, this study investigated some inflammatory factors and has limitations. Thus, in this study, it is recommended to conduct more studies in this field (7). Bisoprolol is also a beta-blocker drug. In an animal study on rats, the effect of this drug on ischemia-reperfusion injury of the heart was investigated. In this study, after the oral administration of bisoprolol, the heart of rats was subjected to ischemia for half an hour and reperfusion for four hours. The results revealed that bisoprolol significantly reduces the rate of cell injury in the heart after ischemia-reperfusion. Bisoprolol also increased cell viability and reduced apoptosis. Moreover, cardiac enzymes in the group receiving bisoprolol decreased compared to the control group. Additionally, this study reported that the protective effect of bisoprolol against injury caused by ischemia-reperfusion is through the PI3K/AKT/GSK3β messenger pathway (8).

Studies have also revealed it affected the production of nitric oxide and its release. Since nitric oxide can reduce hepatocellular apoptosis, oxidative stress, and leukocyte attachment, and improve mitochondrial function, it can be effective in heart injuries (7, 8). Given the effect of bisoprolol in reducing oxidative stress during ischemia-reperfusion injury and also the effect of this drug on some mechanisms proposed for cardiac ischemia-reperfusion injury, this study

investigates the effect of bisoprolol on serum levels of ALP and LDH in liver ischemia-reperfusion model in male rats. ALP is an enzyme found in the blood. It has many forms and helps break down proteins. The liver is one of the primary sources of ALP. The serum level of this enzyme increases when the liver cell is injured. Accordingly, ALP serum level is measured to examine liver injury. However, this enzyme is also found in bones, intestines, pancreas, and kidneys (9).

An experimental study conducted by Balzan et al. (2017) examined the pretreatment effect of metoprolol on liver ischemia-reperfusion injury in pigs. The results showed that this drug does not decrease liver enzymes such as AST, ALT, and ALP (10). In another study by Mr. Hassan et al (2021), the effect of carvedilol on liver ischemia-reperfusion injury in rats was investigated. The results revealed that liver enzymes including AST, ALT, and ALP, as a marker of liver injury, increase significantly after induction of the ischemia-reperfusion model. This study also showed that carvedilol can reduce the rate of liver injury and improve the level of liver enzymes compared to the control group (11). Experimental studies on animals have reported the beneficial effects of some drugs in reducing ischemia-reperfusion injury. However, human studies should also confirm these effects. Hence, researchers are trying to examine new drugs that are effective in ischemia-reperfusion mechanisms. One of the drugs used in this regard is beta-blockers. Limited studies have proven their beneficial effect on the pathways of liver injury caused by ischemia-reperfusion. Bisoprolol is one of the beta-blockers whose role has been identified in reducing the injury caused by ischemia-reperfusion in some organs such as the heart. Most of the studies conducted on beta-blocker drugs, including bisoprolol, have investigated the effect of these drugs on organs such as the heart. Thus, given the effects of bisoprolol in reducing the injury caused by ischemia-reperfusion and considering the significance of surgeries and liver transplant and preventing its complications, this study investigates the effect of bisoprolol on the serum levels of ALP and LDH enzymes in the liver ischemia-reperfusion model in male rats.

Materials and Methods

This experimental study was conducted in the Physiology Laboratory of Yasouj University of Medical Sciences. In this study, 28 white male Wistar rats obtained from the laboratory animal reproduction and maintenance center, Alborz University of Medical Sciences, with a weight range of 180-250 grams, were randomly divided into four groups. The animals were kept under standard thermal conditions of 2±22°C and a 12-hour light cycle. They had free access to standard food and water. Rats were starved 16-18 hours before the experiments. However, they had free access to water. The rats were anesthetized by ketamine and xylazine (75+7.5 mg/kg/ip) (12, 13). The animals were subjected to laparotomy surgery after shaving their abdomen and sterilizing with saline and betadine. Accordingly, an incision of approximately 3-4 cm was made from the middle part of the abdomen. Then, the intestines were carefully removed and kept as far away from the abdominal cavity using cotton swabs as possible until the portal vein and its

accompanying structures appeared. Then, the fourth lobe of the liver was separated from the left lateral lobe to reveal the portal triad (portal vein, hepatic artery, and bile duct). Then, a small vessel clamp was carefully placed around the portal vein and hepatic artery just above the bifurcation of the right lateral lobe to induce IR. The color of the middle and left lobes, which include almost 70% of the liver, changed quickly from the normal red-brownish to pale brown during the induction of ischemia. After clamping the portal vein and hepatic artery, the intestines were returned to the abdominal cavity (14, 15). Thirty minutes after ischemia, the clamp was removed and the abdominal muscles and then the abdominal skin was sutured, and liver reperfusion was performed for 30 minutes (16).

Study groups

1 - Sham group: It received 2 ml Normal Saline (NS) daily through gavage for 7 consecutive days and then surgery without induction of ischemia-reperfusion (IR)

2 - BSP group: It received 10 mg/kg BSP (dissolved in 2 ml of normal saline) through gavage for 7 consecutive days and then surgery without IR induction

3 - IR group: It received 2 ml NS daily through gavage for 7 consecutive days and then surgery and IR induction

4 - IR + BSP group: It received 10 mg/kg BSP through gavage for 7 consecutive days, then surgery and IR induction.

The method of serum preparation

After the IR time, blood was taken from the heart of all four groups. Then, the animals were euthanized by an incision in the diaphragm. The blood obtained from the hearts of the rats was stored in tubes to clot. Then, the clotted blood was centrifuged at 5000 rpm and 37°C for 5 minutes, and the serum of the upper part was removed to measure the desired factors (14).

Measurement of serum levels of ALP and LDH enzymes

The serum levels of ALP and LDH enzymes in the serum of the studied groups were measured by the Man Company kits and with the Selectra biochemical analyzer in the university laboratory.

Data analysis

The obtained data were statistically analyzed in SPSS software. Data are shown as mean ± standard errors of the means. One-Way ANOVA and post hoc tests (LSD or Dannet) were used to compare the means of the groups. $P<0.05$ was considered significant for all data.

Results

As shown in Figure 1, the mean serum concentration of ALP (alkaline phosphatase) in the bisoprolol group did not show a significant change compared to the Sham group (P>0.05). Also, bisoprolol did not cause a significant change in the mean serum concentration of ALP in the BSP+IR group compared to the IR group (P>0.05).

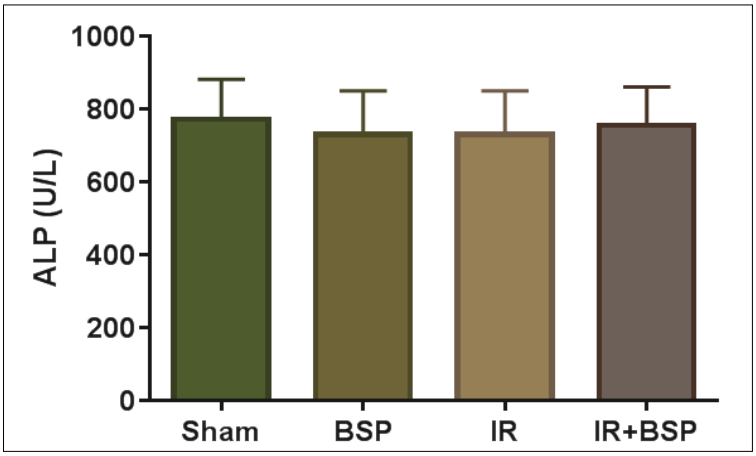

Figure 1: Effects of pretreatment with bisoprolol [10 mg/kg, gavage, 7 days] on serum ALP level in liver ischemia-reperfusion injury

Data were expressed based on mean ± SEM, n=7.

IR ischemia-reperfusion, IR+BSP: ischemia-reperfusion pretreatment with bisoprolol, IR: Ischemia/Reperfusion, BSP: Bisoprolol, ALP: alkaline phosphatase

As shown in Figure 2, the mean serum concentration of LDH (lactate dehydrogenase) in the bisoprolol group did not change significantly compared to the Sham group (P>0.05). Bisoprolol also did not cause a significant change in the mean serum concentration of LDH in the IR + BSP group compared to the IR group (P>0.05).

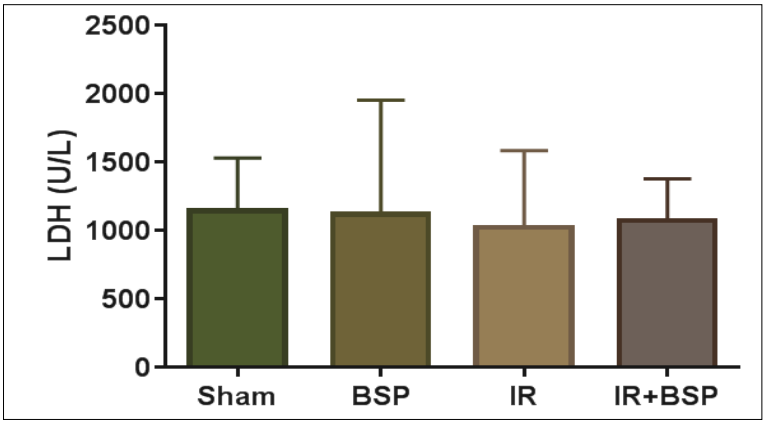

Figure 2: Effects of pretreatment with bisoprolol [10 mg/kg, gavage, 7 days] on LDH serum level in liver ischemia-reperfusion injury.

Data were expressed based on mean ± SEM, n=7.

IR ischemia-reperfusion, IR+BSP: ischemia-reperfusion pretreatment with bisoprolol, IR: Ischemia/Reperfusion, BSP: Bisoprolol, LDH: Lactate dehydrogenase

Discussion

Alkaline phosphatase (ALP) is one of the enzymes found in liver cells. Its concentration in the blood changes during liver injury. Although its measurement is not specific to liver cell injury, it can be a marker of injury. In our study, the serum level of ALP in the bisoprolol group was not significantly different from the "sham" group. Moreover, the serum ALP level in the IR+BSP group was not significantly different from the IR group. In a study by Hassan et al. (11), carvedilol (a non-specific antagonist of alpha and beta-adrenergic receptors) significantly reduced the serum level of ALP in the liver ischemia-perfusion group. Regarding the effect of bisoprolol, no study was found to examine the effect of ALP level on liver injury. Regarding other beta-blockers, the study by Balzan et al. examined the effect of metoprolol in the liver ischemia-reperfusion model in pigs, and similar results were reported (10). The results revealed that metoprolol could not significantly change the level of liver enzymes including ALP compared to the control group. The major limitation of Balzan et al.'s study was that specific markers of liver injury were not investigated, as in our study.

The study by Hassan et al., inconsistent with the results of our study, showed that carvedilol, as a non-specific beta blocker, can reduce the serum level of ALP in the liver ischemia-reperfusion group (11). Thus, the results of the studies can be different depending on the type of drug used, the dosage of the drug, and the duration of ischemia-reperfusion. Our study results regarding the effect of beta-blockers on the serum level of ALP during ischemia-reperfusion were similar to Balzan et al.'s study and contrary to Hassan's study. Lactate dehydrogenase (LDH) is another enzyme that increases during cell injury and destruction. Our study revealed that bisoprolol did not cause a significant change in the serum LDH level in the group that underwent ischemia and reperfusion compared to the IR group. No study was found that directly examine the effect of bisoprolol on LDH serum level in the liver ischemia-reperfusion model in rats. Hence, based on the results of our study, bisoprolol probably cannot significantly change the serum level of LDH in the rat liver ischemia-reperfusion model.

Conclusion

The results of this study showed that pretreatment with bisoprolol at a dose of 10 mg/kg by gavage for seven days before the start of liver ischemia-reperfusion probably cannot significantly change the serum levels of ALP and LDH enzymes. However, these results may change by

changing the dose of the drug used, the type of beta-blocker drug, the method of drug administration, the duration of drug administration, the duration of ischemia-reperfusion, and the type of laboratory animal and other variables, and this issue was observed in the studies mentioned above.

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