**PROTECTIVE EFFECTS OF TIROFIBAN HYDROCHLORIDE, A GLYCOPROTEIN IIB/IIIA INHIBITOR, ON LIVER ISCHEMIA/REPERFUSION INJURY IN RATS**

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**Introduction**

Ischemia reperfusion is common in many medical disciplines. Even though IR is seen in trauma, organ transplantation, myocardial infarction, stroke, shock, burn injury, sepsis and many other clinical conditions, there are still ongoing debates about the development process of IR. Ischemia reperfusion damage occurs differently in various tissues. The rate of liver transplantation for patients with end-stage liver disease has been increasing day by day. The most prominent mechanism that affects the morbidity and mortality of hepatic transplantation is hepatic ischemia-reperfusion injury.[1]

Ischemia reperfusion (IR) consists of a series of processes, which causes cellular and tissue damage. Ischemia is lack of oxygen and metabolic supply to the tissues and failure in clearance of residual metabolites.[2] Reperfusion is the recover of the circulation in the ischemic tissue. Ischemia that occurs as a result of a decrease in the vascular bed is the most common type of cellular damage. Several histological and biochemical changes that are related to the disturbances in protein synthesis, cellular membrane, genetic components of the cell, and oxidative phosphorylation occur in ischemia. The damage is increased even more with the reoxygenation of an organ or tissue, so called reperfusion[3].

**ABSTRACT**

**Aims:** Both ischemia and reperfusion (IR) can cause damages in cells. There are several studies to eliminate the damage. Tirofiban Hydrochloride (TH) is a common glycoprotein IIb/IIia inhibitor and is being used for ischemic disorders for many years. We aimed to determine the therapeutic effects of tirofiban in liver ischemia reperfusion injury model in this animal study.

**Materials and methods:** We induced a 45-minute hepatic ischemia via portal vein, hepatic artery and bile ducts and a 60-minute reperfusion immediately after hepatic ischemia in male albino Wistar rats. One of the groups received intraperitoneal 0.25 mg/ml TH 30 minutes before ischemia and other received intraperitoneal 0.25 mg/ml TH 30 minutes before reperfusion. At the end of the experiment, all animals were decapitated and blood and tissue samples were collected.

**Results:** To evaluate hepatic functions, we assessed serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). In hepatic tissue samples, cataalse (CAT), superoxide dismutase (SOD), and glutathione (GSH) levels were evaluated. We stained hepatic tissue samples with Hematoxylin and Eosin and explored them with a light microscope. Serum AST, ALT, and LDH levels were increased after IR and decreased significantly in the group in which TH was administered. Tissue CAT, SOD, and GSH levels were decreased in IR groups. In TH groups, antioxidant levels were increased when compared to IR groups. Hepatocellular injury that indicates IR-related damage was decreased substantially after TH administration.

**Conclusion:** According to the results of the study, TH decreased the destructive effects of IR. We suggest that TH treatment may can be used in the treatment of hepatic IR damage.

**Key words:** Liver, ischemia reperfusion, Tirofiban Hydrochloride, antioxidants.

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Following the termination of blood flow in ischemia, some metabolic and structural alterations occur in the tissue. Oxidative phosphorylation is decreased at the cellular level and high-energy phosphates such as adenosine triphosphate and creatine levels are decreased\(^4\). Energy storage is emptied and Na\(^+\) K\(^+\) ATPase pumps are inhibited causing an intracellular accumulation of Na\(^+\) and Ca\(^++\) ions\(^5\). Cytotoxic effects are seen due to Ca\(^++\) accumulation in the cell\(^6\).

Clumping of the hepatic artery and portal vein is called as Pringle maneuver. It is used to control bleeding in large liver injuries, transplantation and liver resections. There are studies showing that Pringle maneuver can be used up to 90 minutes in liver resections\(^7\).

Tirofiban is a non-peptide agent which reversibly antagonizes glycoprotein IIb/IIIa receptors. Glycoprotein IIb/IIIa receptors are the major platelet membrane receptors that play a role on platelet aggregation. It blocks platelet aggregation by inhibiting the fibrinogen formation. Several randomized-controlled studies showed that tirofiban blocks platelet aggregation vigorously and prevents arterial thrombosis\(^8, 9\). It is used in treatment of myocardial infarction. There are many studies aimed to reduce the ischemia-reperfusion injury and enhance liver regeneration. In this study we aimed to determine the therapeutic effects of tirofiban hydrochloride (TH) in hepatic IR injury\(^10, 11\).

**Materials and methods**

In total, 32 adult and male Wistar Albino rats weighing between 180 and 300 gr are included in our study, which was carried out in the laboratories of Animal Studies Research Centre, Mustafa Kemal University School of Medicine. The procedures in this experimental study were performed in accordance with the National Guidelines for the USE and Care of Laboratory Animals and approved by the Animal Ethics Committee of Mustafa Kemal University.

All rats were kept under the standard environmental conditions and fed with standard rat feed and water for seven days to let them to adjust to the setting. Rats received 0.5 ml Ketamine (Ketalar 50 mg/ml, Pfizer), 0.1 ml Xylazine HCl (Alfazyne \%, 20 mg/ml, Alfasan International) for anesthesia induction. A midline abdominal incision for laparotomy was made.

Rats were randomly allocated into four equal groups.

**Group A**: (Controls). Portal hilum was explored. After ninety minutes, intracardiac blood and hepatic tissue samples were taken.

**Group B**: (Ischemia/Reperfusion group). Rats were kept in a 45 minute ischemic condition and 60 minutes of reperfusion condition. Following this procedure, intracardiac blood and hepatic tissue samples were taken.

**Group C**: All rats received 0.25 mg per ml intraperitoneal TH 30 minutes before ischemia was initiated. Following 30-minutes of ischemia and 90-minutes of reperfusion, intracardiac blood and hepatic tissue samples were taken and abdomen was closed with silk suture.

**Group D**: Rats were kept in a 30 minutes of ischemic condition and then all rats received 0.25 mg per ml intraperitoneal TH 30 minutes before reperfusion. Following a 90-minute reperfusion, intracardiac blood and hepatic tissue samples were taken and abdomen was closed with silk suture.

Hepatic tissue samples were stored in -850 \(^\circ\)C in an aluminum foil. Frozen samples were thawed in the room temperature and catalase (CAT), superoxide dismutase (SOD), and glutathion (GSH) activities were measured. Blood samples were centrifuged in 4000 rpm for 5 minutes. Plasma was stored in \(-200 \(^\circ\)C. Samples were thawed in the room temperature and Aspartate transaminase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels were measured.

Right lobes of the livers were resected and fixation of the tissue samples was made with 10% formaldehyde. Following that, samples were embedded into paraffin and micrometer thick sections that shows all surface of the liver were prepared. Those samples were stained with Hematoxylin and Eosin. Similar to the study by Sozen et al.\(^12\), we evaluated vacuolization, hyperesinophilia in hepatocytes, and connections between hepatocytes, hemorrhage, and necrosis and polymorphnuclear leukocyte infiltrations and scored them pathologically (Table 1).

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS\(^®\) 11.0 for Windows\(^®\)). Mean and standard deviations were calculated and hypothesis testing was done with an Analysis of Variance test (ANOVA), Turkey HSD and Duncan tests. A P value of < 0.05 was considered for statistical significance.
Results

AST, ALT and LDH levels in IR group were higher than in controls (p< 0.05). The TH before ischemia group and TH before reperfusion group had statistically lower levels in all parameters when compared to IR group (p<0.05).

TH before ischemia group and TH before reperfusion group did not differ significantly in terms of measured parameters (p>0.05)

Tissue levels of CAT, SOD and GSH were statistically lower in IR group than in controls (p<0.05). TH before ischemia group and TH before reperfusion group had significantly higher levels in all tissue parameters when compared to IR group (p<0.05). There were no significant differences in terms of tissue parameters between TH before ischemia group and TH before reperfusion group (p>0.05).

Table 1: Pathological grading of hepatic injury.

| GRADE 0 | Mild or no damage |
| GRADE 1 | Mild damage, focal nuclear pyknosis and cytoplasmic vacuolization |
| GRADE 2 | Moderate-severe; diffuse nuclear pyknosis; cytoplasmic hyper eosinophilia and intracellular bridge loss |
| GRADE 3 | Severe necrosis; split in hepatic cords, hemorrhage and neutrophil infiltration |

Table 2: Descriptive analyses of blood and tissue parameters.

Table 2: Descriptive analyses of blood and tissue parameters.

Hepatocytes were intact and sinusoid and hepatic parenchyma was in normal morphology in the controls (Figure 1). In IR group, there was vacuolar degeneration in hepatocytes, splitting of the hepatic cords, hemorrhage, overt eosinophilia and pyknosis (Figure 2). Less damage was detected in the histopathological examination of TH before ischemia group and TH before reperfusion group (Figure 3-4).
• In total, six livers in IR group had moderate-severe damage (75%) and two livers had mild damage (25%). In TH before ischemia group, five livers had mild damage (62.5%), three livers had no damage (37.5%). IR group were differed from TH before ischemia group significantly in terms of hepatic damage (p = 0.003).

• In IR group, six hepatic tissues had moderate-mild damage (75%) and two had mild damage (25%). In TH before reperfusion group, two hepatic tissues had moderate-severe damage (25%) and six had mild damage (75%). Differences between those two groups were statistically significant (p=0.046) indicating more pathological alterations in IR group.

• In TH before ischemia group, five livers had mild damage (62.5%), three livers had no damage (37.5%). In TH before reperfusion group, two hepatic tissues had moderate-severe damage (25%) and six had mild damage (75%). Differences between those two groups were statistically significant (p=0.029).

Overall, groups receiving TH had less ischemic changes when compared to IR group.

Discussion

Inflammation is the first step of the IR injury that is characterized with a temporary blockage in the tissue circulation and recirculation.

IR that ends up with cellular death or function loss in an organ occurs as a result of a series of pathological processes. Studies showed that outcomes of reperfusion play an important role on these disturbances. Liver resection, trauma-related injury, and hemorrhagic shock are the leading causes of hepatic ischemia. Blood loss during major hepatic surgery is important in terms of morbidity and mortality. Thus, it is important to minimalize blood loss with vascular isolation. The mechanism behind the IR injury is complex and the free radicals that are formed during hepatic damage are thought to be responsible.

Dawson et al. reported that the mitochondria are the principal source of free oxygen radicals (FOR). These radicals, polymorphnuclear leukocytes (PMNL), complement system and endothelial cells are responsible for damage mechanisms. In the biochemical events that are triggered by tissue damage, the antioxidant system is insufficient. It was shown that due to the oxidants, glutathione levels during the ischemia are decreased and inactivation of the enzymes like SOD, CAT are enhanced in the ischemic tissue. Hence, cells become more sensitive against the effects of FOR those are produced during the reperfusion.

Acute thrombotic lesions are important in terms of ischemia. An atherosclerotic plaque should be ruptured or endothelium should be eroded for thrombosis formation. Adhesion and aggregation of platelets are the first steps in thrombosis formation.

GP IIb/IIIa inhibitors are potent platelet inhibitors. They prevent thrombus formation by stopping platelet aggregation in the arterial thrombus and by dissolving the thrombus.

Tirofiban HCl is used in coronary artery disease as an anticoagulant. Some studies showed that it is effective on stent-related thrombosis. There is a limited number of studies indicating the effectiveness of Tirofiban HCl in IR injury. TH is used as intravenous infusion in cardiology practice, however optimal dose in ischemia is still not clear. In the current study, we aimed to show the effects of intraperitoneal dose of 0.25 mg/ml.

Several methods are available to evaluate hepatic functions. Currently, AST, ALT and LDH levels are commonly used.

Yabe et al. found that serum AST and ALT levels are increased in the livers in which IR was applied. They concluded that these increases might be related with the damage that is caused by free radicals, which are formed as a result of ischemia reperfusion.

Inglot et al. found necrosis, sinusoidal widening, and polymorphonuclear leukocyte (PMNL) infiltration in the hepatocytes in IR. They observed also an increase in AST and ALT levels.

Yildirim et al. performed IR in rat livers and found that serum AST and ALT levels were increased.

In the current study we found significantly low levels of AST, ALT and LDH, which are the indicators of hepatic injury in the groups that received TH. It seems that TH decreases the hepatic damage significantly.

In hepatic IR injury, hepatocellular vacuolic degeneration, splitting in hepatic cords, hemorrhage, overt hypereosinophilia, pyknosis, necrosis and widening in sinusoids are observed.

Crockett et al. detected sinusoidal congestion, hepatocellular necrosis, cytoplasmic vacuolization and neutrophil infiltration in hepatic IR.

In the current study, all hepatic tissues in the controls were in normal morphology. IR group had
vacuolic degeneration, splitting of hepatic cords, hemorrhage, overt hypereosinophilia, and pyknosis similar to previous reports. On the other hand, there was a decrease in IR-related damage in hepatic tissue samples of groups in which TH was administered.

GSH levels were significantly lower in IR group than in controls. The group, in which TH was administered before reperfusion, had increased levels of GSH. Even though there was a similar increase in the group, in which TH was administered before ischemia, it was not statistically significant. We conclude that TH administration before reperfusion increases GSH levels which is a more effective approach to protect the tissue from ischemic damage.

Similar to previous studies, tissue SOD levels were lower in IR group than in the controls. SOD levels were increased when TH was administered before reperfusion. Even though there was an increase in the group, in which TH was administered before ischemia, it was not statistically significant.

It can be concluded that TH administration before reperfusion may help to reduce FOR after reperfusion and this reduction increases the level of antioxidant need. These changes are more prominent when TH is administered before reperfusion rather than before ischemia.

We found decreased CAT levels in IR when compared to the controls. This finding is in line with previous publications. CAT levels were significantly higher in the rats to whom TH was administered before reperfusion than in IR group. Similar to GSH and SOD results, the group that received TH before ischemia had higher levels of CAT than IR group, although this difference was not significantly different.

In conclusion, serum AST, ALT and LDH levels were increased due to the IR-related hepatic damage.

Administration of TH before ischemia and reperfusion decreases the tissue damage and therefore serum AST, ALT and LDH levels.

Administration of TH before ischemia and reperfusion decrease the damage and increases the levels of GSH, CAT and SOD which are used for the neutralization of FOR.

Tirofiban Hydrochloride is used as a platelet aggregation inhibitor in clinical practice. As reported in the current study, it also protects hepatocytes against IR-related injury.

References


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