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Original Article

The Effect of Galantamine on Liver Function in Hepatic Ischemia/Reperfusion Injury in Rats

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Abstract

Background: Hepatic ischemia-reperfusion (IR) injury may affect different biological and functional roles of the liver. The present study aims to evaluate the effects of galantamine as a cholinergic agent on the acute and late phases of IR. **Materials and Methods:** Forty rats were randomly allocated to eight groups (n=5): IR groups were subjected to 90 min ischemia followed by 4 and 24 hours reperfusion to induce acute and late phases of IR, respectively, two groups received pre- or post-treatment dose(s) of galantamine in the acute phase of IR, two groups received pre- or post-treatment dose(s) of galantamine in late phase of IR, and two sham-operated groups. Blood samples were taken, and ALT, AST, ALP, and LDH were measured to evaluate the liver function. **Result:** Pre-ischemic treatment with galantamine decreased the levels of ALT, AST, and ALP in the acute phase of IR, unlike the late phase (P-value<0.05). Post-ischemic treatment with galantamine in the acute phase of IR was decreased all enzymes (P-value<0.05). Unlike the latter, treatment with galantamine in the late phase of IR was increased these values. **Conclusion:** We concluded that pre- and post-ischemic treatment with galantamine could be ameliorated hepatic IR injury, in the acute phase versus the late phase, which should be further studied in detail.

Keywords: Galantamine, Ischemia, Reperfusion, Liver function, Rat

Introduction

Hepatic ischemia-reperfusion (IR) injury is a phenomenon that occurs in clinical conditions such as, liver resection, liver transplantation, trauma, other surgical procedures, and shock (1, 2). A network of intra- and extra-hepatic mechanisms are involved in the pathophysiology of IR injury. There are two separate phases in reperfusion liver lesions. The early phase is associated with hepatocellular damage during 3-6 h after reperfusion (re-oxygenation) and the main event during this phase is the activation of Kupffer cells and the late phase, which reaches a peak 18-24 h after reperfusion with a neutrophil infiltration (1). Injury to the liver caused by IR has been shown with an increased rate of acute liver failure/ graft rejection and chronic liver dysfunction after liver transplantation. Several protective strategies are used for minimizing liver IR damages like pharmaceutical interventions (1, 2). Acetylcholinesterase inhibitors, which include the drugs, galantamine, rivastigmine, and donepezil act by blocking the enzyme acetylcholinesterase and acetylcholine (Ach) (3). Galantamine is considered for the treatment for Alzheimer disease, protect endothelial cells, and vascular dementias. Galantamine has significant anti-inflammatory effects and stimulates the cholinergic anti-inflammatory pathway, which inhibits cytokine production (4). In this pathway, Ach interaction with an α-7 subunit of nicotinic Ach receptors leads to the inhibition of inflammation (5). Hepatic stellate cells including nicotinic Ach receptors subunits with α-7 subtype. In addition to it, is explained that the OH group of galantamine is a scavenger for reactive oxygen species (ROS), so exhibits antioxidant activity (6). As with sepsis and acute liver failure, hepatic IRI is associated with immune system activation. In previous studies, demonstrated the protective effects of the cholinesterase inhibitors in sepsis and acute liver failure by reducing proinflammatory cyto-

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kines and oxidative stress, and increasing anti-inflammatory cytokines and antioxidant level (7, 8). Because inflammation, endothelial cells dysfunction, and oxidative stress play important roles in the pathophysiology of hepatic damages followed IR, the present study was designed to evaluate the effects of pre- and post-ischemic administration of galantamine on liver function in the acute and late phases of hepatic IR injury.

Materials and Methods

Drug

Galantamine hydrobromide (GHB) was purchased from a commercial source (Tehran Darou CO, Iran) and dissolved in distilled water.

Animals

Adult male albino Wistar rats weighing 250-300g were obtained from the faculty of veterinary medicine, Shahid Chamran University of Ahvaz and used in this study. The animals were group-housed with 12-hour light-dark cycle in temperature of 22±2°C with 50±10% relative humidity and fed a standard laboratory diet. Rats were fasted overnight for at least 16 hours prior to the experiments, but access to water *ad labium*. This study was conducted according to all the ethical codes of working on experimental animals approved by the Ministry of Health and Medical Education. Rats were randomly divided into eight experimental groups (n=5). Two sham-operated groups (sham 4h and sham 24h) endured the surgical process without any further manipulation. Two groups underwent 90 min ischemia followed by 4 and 24-hour reperfusion to induce and represent acute and late phases of hepatic IR (IR 4h and IR 24h). Two groups received GHB (3mg/kg, intraperitoneal. i.p) (9, 10) at 2h before the ischemia followed by 4 and 24-hour reperfusion (GHB3+IR 4h and GHB3+IR 24h). One group received a single injection of GHB (3mg/kg, i.p) at 1h time of reperfusion and sacrificed 4h after reperfusion (IR 4h+ GHB3) and other group received repeated dose of GHB (3mg/kg, i.p) at 1, 6, 12 and 18h of reperfusion and sacrificed 24h after reperfusion (IR 24h+ GHB3). For better understanding, the summarized information is presented in Figure-1.

In vivo (lobar) model of IR

Each animal was anesthetized by injection of ketamine (60 mg/Kg, i.p) plus xylazine (10 mg/Kg, i.p) (1) and the body temperature was maintained at 37°C throughout the anesthesia using heating pads. A midline laparotomy was performed, and the median and left lobes of the liver were gently taken out of the abdominal cavity. Partial ischemia was achieved by clamping the blood vessels of the portal vein and hepatic artery and

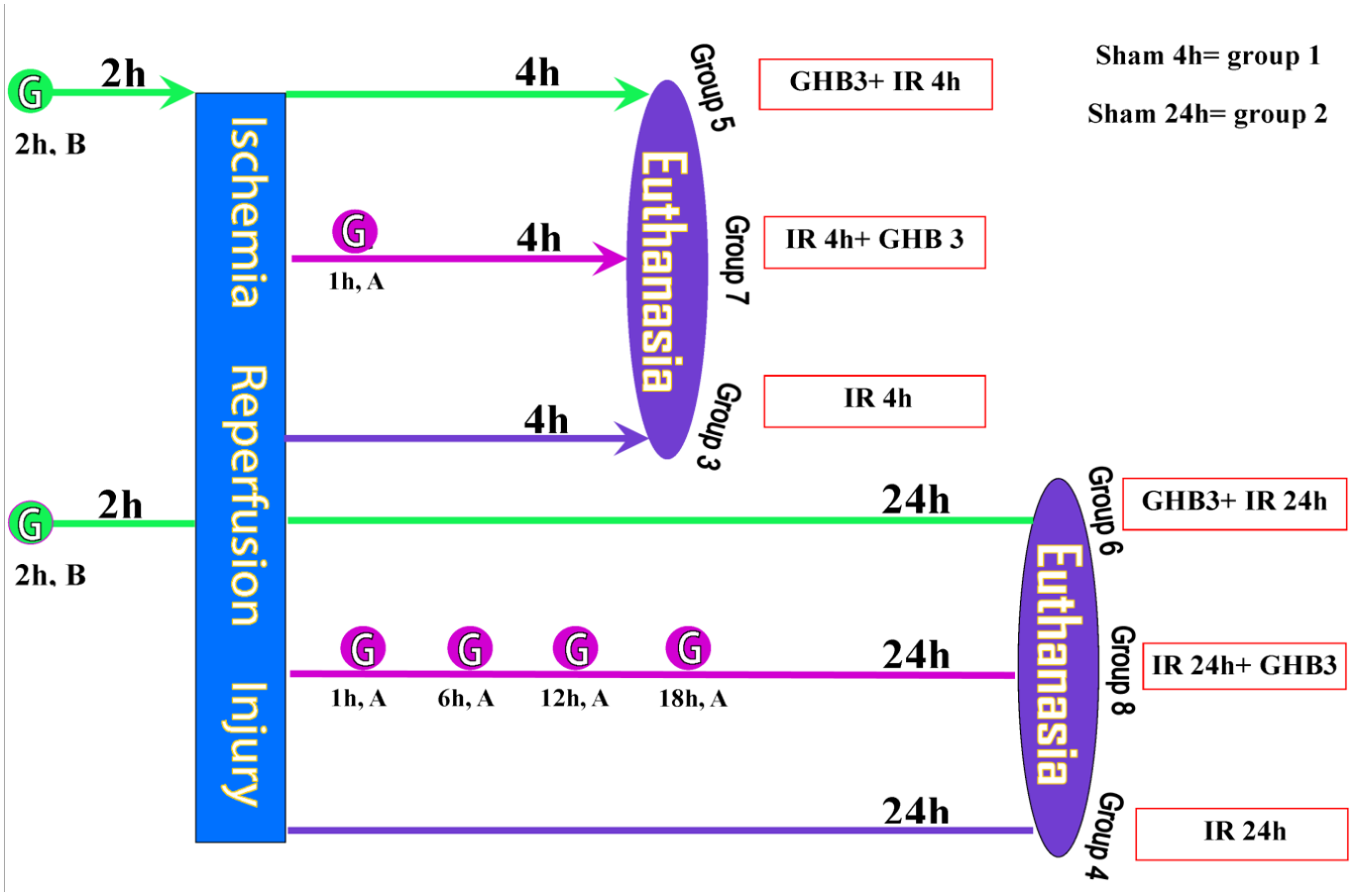

Figure 1. The schematic diagram of experimental processes.
G: Galantamine (GHB). **2h, B**: GHB administration 2 hours before IR. **1h, A**: GHB administration 1 hour after IR. **6h, A**: GHB administration 6 hours after IR. **12h, A**: GHB administration 12 hours after IRI. **18h, A**: GHB administration 18 hours after IR.

bile duct supplying the median and left lateral hepatic lobes for 90 minutes. Clamping induced an immediate discoloration of the hepatic lobes, which is reported to produce partial liver ischemia. The right lobe remained perfused to prevent intestinal venous congestion. Upon the release of the clamp, reperfusion was commenced, and the blood flow was continued for different times. Sham-operated control animals underwent the same surgical procedure, except that the vascular occlusion was omitted. Muscular and cutaneous tissues were then stitched during reperfusion. Blood samples were obtained after different times of reperfusion. The serum was separated by centrifuging the blood (4000 rpm 10 min) and then kept into -20°C until analysis.

Liver function assessment tests

The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were determined by standard spectrophotometric procedures using commercially available kits (Pars Azmon Co. Karaj, Iran) for Hepatocellular damage assay after IR and treatment with GHB.

Statistical analysis

Data were analyzed using SPSS 16 package program. The results are presented as mean± SEM. Statistical analysis was performed by one-way ANOVA followed by post hoc Tukey test. P-value <0.05 was considered statistically significant.

Result

The serum levels of ALT, AST, ALP, and LDH in the animals exposed to the acute phase of hepatic IRI (IR 4h) markedly increased compared to the sham-operated groups (P-value< 0.05). These values maintained high during the late phase of hepatic IR (IR 24h) compared to those in the sham-operated control animals (sham 24h). There were significant changes in enzyme release in pre and post-ischemic treatment with GHB groups, compared to IR and sham-operated groups (P-value< 0.05).

Pre-treatment effects of GHB on liver function with IRI

Pre-treatment with GHB before liver ischemia decreased the level of ALT, AST, and ALP in the animals exposed to the acute phase of hepatic IR (GHB3+IR

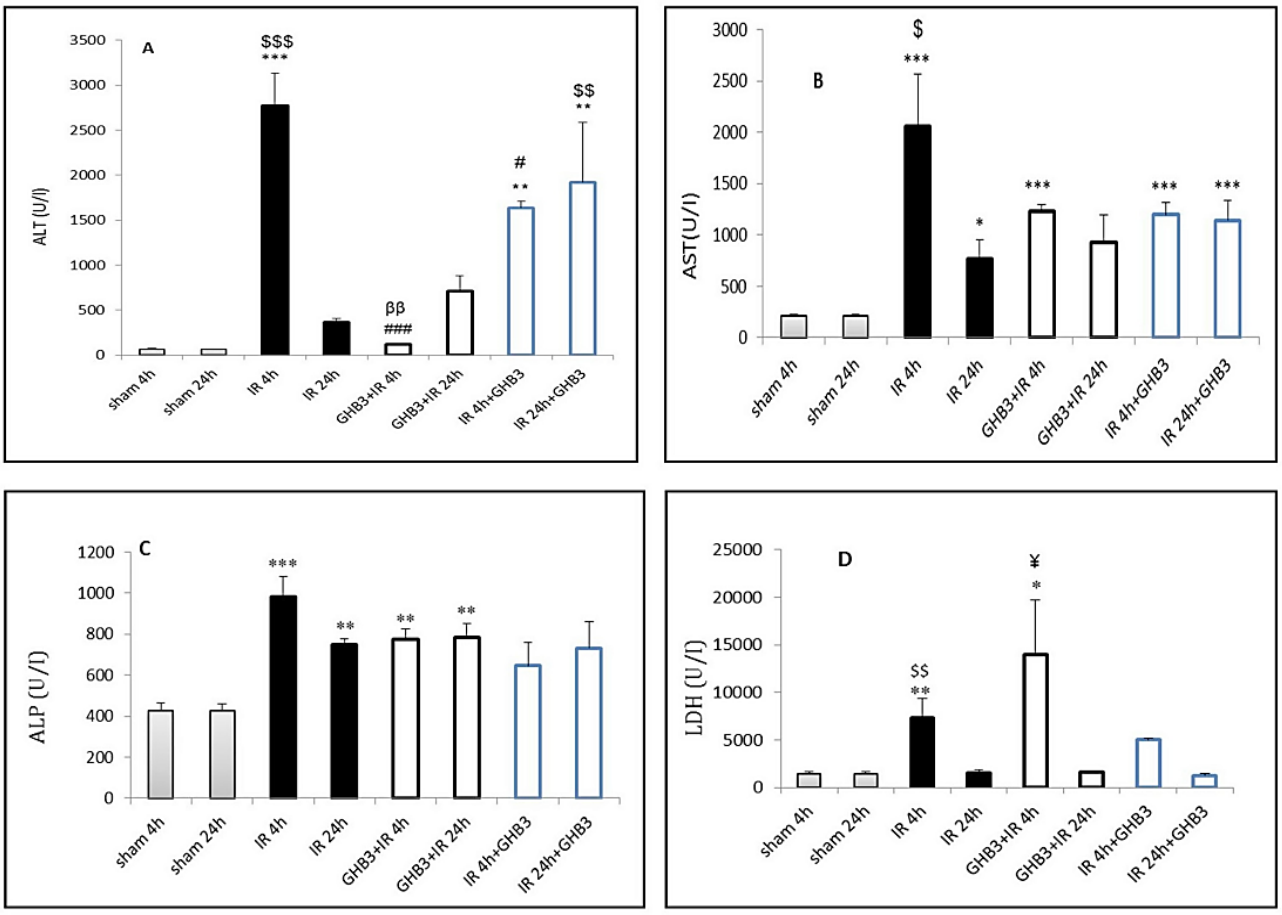

Figure 2. Changes in serum ALT (**A**), AST (**B**), ALP (**C**), and LDH (**D**) following 4 or 24 h of reperfusion in liver IR groups and effects of GHB on liver enzymes. Results are presented as the mean±S.E.M. Statistical analysis was performed by one-way ANOVA using post hoc Tukey test. P-value<0.05 was considered statistically significant. *: indicates significant differences vs the sham group (*:P-value <0.05, **: P-value <0.01, ***: P-value <0.001). #, ###: indicate significant difference vs IR4h group (#:P-value <0.05, ###: P-value <0.001). $: indicates significant differences vs IR24h group ($:P-value <0.05, $$: P-value <0.01, $$$: P-value <0.001). ¥: indicates significant differences vs. GHB3+IR24h group (¥:P-value <0.05). ββ: indicates significant differences vs. IR4h+ GHB3 group (ββ: P-value <0.01).

4h), unlike late phase group (GHB3+IR 24h), compared to IR 4h and IR 24h groups (P-value < 0.05).These levels increased compared to the sham-operated groups.

Post-treatment effect of GHB on liver function with IR injury

Post-ischemic treatment with GBH in the animals exposed to the acute phase of IR (IR 4h+ GHB3), were decreased all enzymes than IR4h group, but just ALT was significant (P-value< 0.05). These levels increased compared to the sham 4h group (P-value< 0.05). Unlike the latter, compared to IR 24h group, post-ischemic treatment with GHB in the animals exposed to the late phase of IR (IR 24h+ GHB3) at different times, were increased the levels of ALT and AST but a change of ALT was significant (P-value< 0.05). In addition to all liver enzymes levels, except LDH were increased than sham-operated animals (sham 24h) that changes of ALT and AST were significant (P-value< 0.05, Figure-2).

Discussion

Liver damage by IR is a global process that affects several pathways, both cellular and molecular (1). IR can be divided into two distinct phases. The early or acute phase is associated with hepatocellular damage during 3-6 h after reperfusion (re-oxygenation) is associated with the free radical generation with Kupffer cell and T-lymphocyte activation. Previous studies suggest that the burst of ROS generated after reperfusion may contribute to the initiation of post-ischemic liver injury and inflammatory infiltration. The late or sub-acute phase, which reaches a peak 18-24 h after reperfusion is accompanied by a massive neutrophil infiltration (1, 11).

Compare to all liver injuries, ischemia, and toxic injuries lead to the highest amount of aminotransferase enzymes (12), and the extent of liver injury in IR is normally measured by levels of serum liver enzymes, such as ALT, AST, ALP, and LDH (2). ALT is found in many tissues, especially the liver that its increase is a sign for hepatocellular injury (13). AST is found in metabolic activating tissues like kidney, brain, red blood cells, skeletal muscle, heart, and liver also destroying the cells lead to AST releasing (14). Toxic liver injury and ischemia cause elevated AST level (15). Zone three of hepatic acinus has more mitochondria and higher AST concentration, so their damage increases AST level. In ischemia, AST reaches to peak before ALT (12). ALT and AST are found in liver parenchyma cells, but ALT is more specified for the liver than AST it means that ALT acts as a definite indicator of liver inflammation, but AST can be elevated in the other tissues injuries like myocardial infarction (16). High ALP is found in various tissues, and its increase can be due to high osteoplastic activity. Its elevation is related to many organs disorders like liver disease, hepatitis and bile ducts obstruction, so that elevated level of ALP is related to hepatobiliary diseases (17). As it is located on liver canaliculus, its amount is increased, especially in bile duct obstruction. LDH is an intracellular enzyme, found in several tissues like liver and blood cells, and its amount increases in tissue damage (18). It is produced at the terminal stage of glycolysis in the anoxia/hypoxia condition, and its translation changes between aerobic and anoxic condition (19). Ischemia results in anoxia or hypoxia, so after ischemia induction certainly, LDH level is increased. Our results demonstrated that the serum levels of ALT, AST , ALP, and LDH in acute phase of hepatic IR (IR 4h) markedly increased compared to the sham-operated groups (P-value< 0.05) and these values maintained high at late phase of hepatic IR (IR 24h) compared to those in the sham-operated control animals (sham 24h). On the other hand, pre and post-ischemic treatment with GHB in the acute phase of IRI, unlike the late phase decreased the levels of ALT, AST, and ALP. Unlike the latter, Post-ischemic treatments with GHB in the late phase of IRI have increased these values. It has been shown that heme oxygenase-1 (HO-1) protects the liver against IR injury via modulation of pro-inflammatory factors. HO-1 is an enzyme that degrades hem and is unregulated in oxidative stress (20) and its induction in acute and chronic hepatic inflammations that improved liver functions (21). Activation of the cholinergic anti-inflammatory pathway leads to nicotine production, which reduces IR damages through heme oxygenase-1 (22). Following IR, the HO level elevates and nicotine increases this augmentation, which leads to inhibition of the inflammatory response, decreased ALT, and enhanced ROS scavengers (22). Moreover, it has shown that neostigmine, as an AChEI, improves liver function after acute liver failure is mediated via the α -7nAch receptor with anti-inflammatory and antioxidant effects (23). On the other hand, the formation of ROS has been shown to activate redox-sensitive transcription factors, such as nuclear factor (NF)- kappa B (NF-κB) and activator protein (AP)-1 that play a major role in the pathophysiology of IRI by activating inflammatory cascades leading to organ damage. NF-κB induces the synthesis of iNOS (inducible NO synthase), adhesion molecules, chemokines, and cytokines (TNF-α). NF-κB is activated during two different stages of IR, with different actions: at an early stage (from 30 min to 3 h of reperfusion), it induces an increase in the expression of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α). At a later stage (9-12 h after reperfusion), it acts as an anti-inflammatory agent (24). It is demonstrated that the protective effects of galantamine in some inflammatory states require NF-κB activation, iNOS expression, and HO-1 activity although its anti-inflammatory and antioxidant effects are more pronounced in the acute phase of hepatic IR (3). Moreover, it has been shown that galantamine treatment of obese mice, suppressed serum ALT levels, and liver enlargement to levels determined in mice (5). In conclusion, GHB may be useful to prevent ischemic

liver damage at the early or acute phase of IR unlike late phase and more evaluations need to declare that GHB can be used for prophylaxis and protect liver damage in hepatic IR injury.

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Conflict of interest

The authors declare no conflict of interest.

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