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Anti-Oxidative Effect of Simvastatin in Liver and Lung Tissue after Hepatic Ischemia/Reperfusion in Rat

[1]B. Hajipour, [2]M.H. Somi, [3]F. Dibazar, [4]N.A. Asl and [5]A.M. Vatankhah

In this research we studied simvastatin effect on liver and lung tissue anti-oxidant enzymes, 24 h after hepatic I/R. Male Wistar rats were randomly assigned into three groups of fifteen animals in each group. Group 1, the sham group, underwent laparotomy but did not experience I/R; group 2, the I/R group, underwent ischaemia for 30 min and were reperfused for 24 h; Group 3 underwent ischaemia and reperfusion like group 2 but also received 10 mg kg^{-1} simvastatin dissolved in water by oral gavage for three days before the operation. In animals subjected to hepatic I/R port triad clamped for 30 min following by 24 h reperfusion. Data showed that GPx and SOD levels in liver and lung tissue in I/R+simvastatin group were higher than I/R group significantly ($p<0.05$). MDA level in I/R group was significantly higher than I/R+simvastatin group in liver and lung tissue significantly ($p<0.05$). As simvastatin is a safe drug and result of our previous research and also this research show the efficacy of simvastatin pretreatment in decreasing liver and lung oxidative stress after hepatic I/R, so it may be useful in attenuating hepatic I/R injury and it may be effective for performing safer hepatic surgeries.

Key words: Simvastatin, liver, ischemia/reperfusion, lung, rat-injury

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For further information about this article or if you need reprints, please contact:

Babak Hajipour
Faculty of Medicine,
Islamic Azad University,
Tabriz Branch, Tabriz,
Post Box 51385-3633, Iran

[1]Faculty of Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran
[2]Liver and Gastroenterology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Department of Anatomy, Faculty of Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran
[4]Department of Physiology, Tabriz University of Medical Sciences, Tabriz, Iran
[5]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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INTRODUCTION

Hepatic ischemia and reperfusion (I/R) include interruption of hepatic blood flow that is named ischemia phase and then increase in hepatic blood flow because of improved hepatic blood flow, in which is called reperfusion phase. Hepatic I/R usually occur in cardiac and hemorrhagic shock and in hepatic surgeries (Jawan *et al*., 2003). For suppression of blood loss, clamping of the portal triad (inflow occlusion), also called pringle maneuvre, is used during liver surgery. However, the risk of I/R injury of the liver is increased under these conditions (Dünschede *et al*., 2006; Pringle, 2008). Various mechanisms involve in hepatic I/R injury. Activated kupffer cells release Reactive Oxygen Species (ROS) (Jaeschke and Farhood, 1991) and pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) (Colletti *et al*., 1990), in which triggers inflammatory pathways and cause neutrophil accumulation and enhances cellular apoptosis and tissue damage Ma and Ma (2008). Free radicals cause lipid peroxidation of the cellular membranes and malondialdehyde is generated. The injury of the cellular membranes destroys the homeostasis of hepatocytes, which leads finally to cellular damage and liver dysfunction (Molina *et al*., 2003; Váli *et al*., 2006). In addition to liver injury, hepatic I/R causes distant organ injury including lung (Takamatsu *et al*., 2006). The organ dysfunction that accompanies this condition is generally associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration and parenchyimal cell dysfunction and necrosis (Kaçmaz *et al*., 2005). Several factors have been reported to be responsible for it. Key pathophysiological events that occur in the pulmonary microvasculature take place at the interface of the alveolar capillary membrane, where sequestered inflammatory cells and mediators (both local and humoral) induce oxidative stress (Lin *et al*., 2006) and (Yuan *et al*., 2005).

Statins are cholesterol-lowering agents with proven efficacy and safety and are widely used for prevention of cardiovascular disease. Statins action is because of their effect on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step of the cholesterol synthesis in the liver and other tissues (Corsini *et al*., 1995; Işeri *et al*., 2007). By blocking cholesterol synthesis in the liver, statins activate hepatocyte Low-Density Lipoprotein (LDL) receptors and produce reductions in circulating LDL cholesterol with improvements in cardiovascular risk by retarding atherosclerosis in all major arteries. In addition to beneficial effects on lipid profiles, statins also improve endothelial function, enhance cellular anti-oxidant levels and have anti-inflammatory, anti-platelet aggregation and anti-thrombotic effects (Farooqui *et al*., 2007; Cowled *et al*., 2007). Previous studies indicate that simvastatin can decrease I/R injury in different organs including brain (Cakmak *et al*., 2007), kidney (Todorovic *et al*., 2008), heart (Szárszoi *et al*., 2008) and liver (Dibazar *et al*., 2008), but exact mechanisms of simvastatin protection against hepatic I/R injury are not known well.

Our previous study showed that simvastatin attenuates hepatic I/R injury and decreases cellular damage and number of apoptic cells in liver and lung (Dibazar *et al*., 2008). We have shown that simvastatin pretreatment decreases TNF-α level and hepatic transaminases after hepatic I/R, we hypothesized that protective effect of simvastatin in hepatic I/R induced liver and lung injury may be due to its anti-oxidant properties. In this research we measured superoxide dismutase (SOD) and glutathione peroxidase (Gpx) as anti-oxidant enzymes and malondialdehyde (MDA) as an index of lipid peroxidation and oxidative injury in liver and lung tissue 24 h after hepatic I/R.

MATERIALS AND METHODS

Animals: Male wistar rats were purchased from Tabriz Medical University Animal Care Center and had free access to laboratory chow and water and housed in plastic cages and they were kept according to university guidelines for care and use of laboratory animals. This project was conducted from 2008 to 2009. Animals were randomly assigned into three groups of fifteen animals in each group. Group 1, the sham group, underwent laparotomy but did not experience I/R; group 2, the I/R group, underwent ischaemia for 30 min and were reperfused for 24 h; Group 3 underwent ischaemia and reperfusion like group 2 but also received 10 mg kg^{-1} simvastatin dissolved in water by oral gavage for three days before the operation.

Surgery procedure: Total hepatic ischemia model performed for induction of ischemia. Rats anesthetized with ketamin (50 mg kg^{-1}) and xylazine (10 mg kg^{-1}), abdomen wall shaved and prepared with betadaine, midline incision performed and portal triad clamped for 30 min, after 30 min ischemia clamps removed and liver reperfused for 24 h, after removing the clamps the abdomen wall closed with sutures (Dibazar *et al*., 2008). After surgery process animals had free access to food and water. Twenty four hours after surgery animals

anesthetized and liver and lung tissue excised and preserved in -20C until biochemical analysis for measuring tissue GPx, SOD and MDA level.

Assay of antioxidant enzymes: In order to measure anti-oxidant enzyme activity, the tissue samples were homogenized in 1.15% KCL solution. SOD activity in tissue was determined by using xanthine and xanthine oxidase to generate superoxide radicals which then react with 2-(4-iodophenyl)-3-(4-nitrojenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was then measured by the degree of inhibition of this reaction (Ransod, Randox Laboratories Ltd. United Kingdom). Results obtained SOD Unit/mg protein (Paoletti *et al*., 1986).

Gpx activity in tissue was measured using the method described by Paglia and Valentine (1967). GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidised glutathione is immediately is converted to reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. (Ransod, Randox Laboratories Ltd. United Kingdom).Results obtained GPx Unit/mg protein.

Tissue MDA level: MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) method (Kaya *et al*., 2004).

Statistical analysis: Comparison among groups was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's test. Significance was set at $p<0.05$; data are reported as Mean±SD.

RESULTS

Gpx and SOD: Hepatic I/R decreased GPx and SOD levels both in liver and lung tissue significantly ($p<0.05$, Table 1 and 2). Results showed that GPx and SOD levels were higher in simvastatin + I/R group comparing to I/R group significantly in liver tissue, 24 h after I/R induction ($p<0.05$, Table 1).

The results also showed that GPx and SOD levels were higher in simvastatin + I/R group comparing to I/R group significantly in lung tissue 24 h after I/R induction ($p<0.05$, Table 2).The levels of GPx and SOD were lower in liver tissue of rats in I/R+simvastatin group comparing to sham group($P<0.05$, Table 1). The levels of GPx and SOD were lower in lung tissue of rats in I/R+simvastatin group comparing to sham group ($p<0.05$, Table 2).

MDA level: Hepatic I/R increased MDA level both in liver and lung significantly ($p<0.05$, Table 1, 2).

Table 1: Level of GPx, SOD and MDA in rat liver tissue after 24 h hepatic I/R

Levels	Sham	Hepatic I/R	Hepatic I/R+simvastatin
SOD (U/mg/tissue)	4.21±0.36	2.41±0.35	3.20±0.35
Gpx (U/mg/tissue)	3.40±0.39	1.20±0.35	2.40±0.35
MDA (nMol mL^{-1})	0.81±0.24	2.80±0.20	1.60±0.30

The values are shown as a Mean±SD for rats in each group and difference of ($p<0.05$) considered significant. GPx: Glutathione peroxidase, SOD: Superoxide dismutase, MDA: Malondialdehyde

Table 2: Level of GPx, SOD and MDA in rat lung tissue after 24 h hepatic I/R

Levels	Sham	Hepatic I/R	Hepatic I/R+simvastatin
SOD (U/mg/tissue)	3.70±0.26	1. 80±0.45	2.50±0.41
GPx (U/mg/tissue)	2.70±0.39	1.28±0.58	1.92±0.26
MDA (nMol mL^{-1})	0.83±0.38	2.40±0.55	1.70±0.44

The values are shown as a Mean±SD for rats in each group and difference of ($p<0.05$) considered significant. GPx: Glutathione peroxidase, SOD: Superoxide dismutase, MDA: Malondialdehyde

Measurement of MDA level indicated that its level in liver tissue was higher in I/R group than simvastatin+I/R group significantly ($p<0.05$, Table 1). Results also showed that MDA level in lung tissue was higher in I/R group comparing to I/R+simvastatin group significantly ($p<0.05$). MDA levels were higher in liver and lung tissue of rats in I/R+simvastatin group comparing to sham group significantly ($p<0.05$) (Table 1, 2).

DISCUSSION

The objective of this study was to evaluate the anti-oxidant effect of simvastatin administration in preventing the damage associated with liver I/R in liver and lung tissue. It was previously understood that increasing production of oxygen radicals and post ischemic damage of the liver have a two phase time course: an initial phase of injury (roughly within the first 2 h after reperfusion) and a late phase, from approximately 3 to 24 h of blood reflow (Colletti *et al*., 1990; De Tata *et al*., 2005). In reperfusion phase there is an overproduction of oxygen radicals in activated Kupffer cells (De Tata *et al*., 2005) and in the mitochondria of hepatocytes and endothelial cells, where oxygen reflow encounters highly reduced respiratory chains (Jaeschke *et al*., 1990).

Oxidative stress play an important role in inducing tissue injury after I/R. Oxidative stress is generally defined as an imbalance that favors the production of ROS over their inactivation by antioxidant defense systems; however, the precise mechanisms by which ROS produce cellular injury remain elusive. The majority of ROS are products of mitochondrial respiration. The one-electron reduction of molecular oxygen produces a relatively stable intermediate, the superoxide anion radical, which can be regarded as the precursor of most ROS in biological systems. Despite their content of various antioxidants and detoxifying enzymes, the mitochondria

appear to be the most powerful intracellular source of ROS. The dismutation by superoxide dismutase results in hydrogen peroxide production. Subsequent interaction of and H_2O_2 in a Haber-Weiss reaction, or iron- or cupper-driven cleavage of H_2O_2 in a Fenton reaction, can generate the highly reactive hydroxyl radical (Orrenius *et al*., 2007). For attenuating devastating effects of ROS, organisms have developed a variety of antioxidant defense systems, especially the endogenous antioxidant enzymes system including SOD and GPx (Yuan *et al*., 2005).

MDA is a sensitive index to show lipid peroxidation. Lipid peroxidation induces both structural and functional injury to the cell organelle membranes. Reactive oxygen species-induced lipid peroxidation plays important role in the extent of tissue injury.

The overall process of lipid peroxidation consists of three stages: initiation, propagation and termination. Once formed, peroxyl adicals (ROO·) can be rearranged via a cyclisation reaction to endoperoxides (precursors of malondialdehyde) with the final product of the peroxidation process being malondialdehyde (MDA). MDA is mutagenic in bacterial and mammalian cells and arcinogenic in rats. Present results showed that simvastatin treatment decreased liver and lung tissue MDA contents significantly (Valko *et al*., 2006). One of the most effective intracellular enzymatic antioxidants is SOD. SOD is an important antioxidant enzyme in cells and catalyzes the conversion of superoxide ions into oxygen and hydrogen peroxide. SOD destroys $O_2^{\bullet-}$ with remarkably high reaction rates, by successive oxidation and reduction of the transition metal ion at the active site in a Ping-Pong type mechanism (Valko *et al*., 2006). GPx is glutathione related enzyme, which can catalyze the synthesis of GSH to ease the burden of lipid peroxidation. GPx acts in conjunction with the tripeptide glutathione (GSH), which is present in cells in high (micromolar) concentrations. The substrate for the catalytic reaction of GPx is H_2O_2, or an organic peroxide ROOH (Valko *et al*., 2006). Present study showed that liver and lung tissue GPx and SOD level 24 h after hepatic I/R were higher than I/R group and simvastatin maintained cellular antioxidant enzymes levels significantly.

CONCLUSION

Present results showed that simvastatin attenuated hepatic I/R induced liver and lung tissue anti-oxidant enzymes (SOD and GPx) suppression and decreased tissue MDA level as an index of oxidative injury and final product of lipid per-oxidation. As simvastatin is a safe drug and result of our previous research and also this research show the efficacy of simvastatin pretreatment in decreasing liver and lung injury after hepatic I/R, so it may be useful in attenuating hepatic I/R injury and it may be effective for performing safer hepatic surgeries.

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