EXPERIMENTAL STUDY

The protective effects of dexmedetomidine on hepatic ischemia reperfusion injury

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Abstract: *Objective:* The aim of this study was to evaluate the effect of dexmedetomidine (100 μg/kg-ip) on liver ischemia and reperfusion (I/R) in rats.
Methods: Twenty-four Wistar Albino rats were separated into three groups as control (C), ischemia-reperfusion injury (I/R) and dexmedetomidine group (I/R-D). Ischemia was induced with portal clampage for 45 minutes and reperfusion period was 45 minutes after declampage. Group I/R-D was received dexmedetomidine 100 μg/kg i.p. 30 min before portal clampage. Thiobarbutiric Acid-Reactive Substances (TBARS), glutathioneS-transferase (GST), superoxide dismutase (SOD), Catalase (CAT), and Paraoxonase 1 (PON-1) were investigated in blood samples. Also HSP60 and p53-positive hepatocytes were counted under ImageJ image analysis program.
Results: All parameters, except GST levels, were significant between the groups (p < 0.05). Although HSP60 expression was significantly increased between I/R, I/R-D and C groups there were no significant differences between I/R-D and C (p = 0.443). On the other hand, p53 expression was also significantly increased between I/R, I/R-D and C groups At the same time, there were no significant differences between I/R-D and C groups (p = 0.354).
Conclusion: All the results suggest that dexmedetomidine has beneficial effects on liver ischemia/reperfusion stress *(Tab. 1, Fig. 2, Ref. 49).* Text in PDF *www.elis.sk.*
Key words: dexmedetomidine, ischemia reperfusion, HSP60, p53, rat.

Hepatic ischemia – reperfusion (I/R) injury is a common problem in trauma, hepatic surgery, and transplantation. Previous clinical and experimental studies investigated several aspects of this clinical entity (1–4). Hepatic I/R injury is a result of a complex of different mechanisms including both cellular and molecular elements such as leukocytes, TNFα, NO, NFkaB, and the others (2). Oxidative stress, free radical formation and lipid peroxidation play important roles in the development of I/R injury (5).

Oxidative stress occurs particularly in reperfusion after ischemia. During this process proinflammatory cytokines are synthesized and cell adhesion molecules are activated. Thus the inflammatory response is increased by oxidative stress. The antioxidant system has an important role in protection from the damage of oxidative stress (5–9).

Malondialdehyde (MDA) is an intermediate product of lipid peroxidation and is used for assessment of tissue injury attributable to free radicals produced by ischemia/ reperfusion (10).

Different apoptotic pathway regulators including p53 gene which is one of the most important tumor suppressor genes may play important roles to protect tissue from irreversible DNA damage (11).

Dexmedetomidine, a selective and potent α2- adrenoceptor agonist, was approved by the U.S. Food and Drug Administration in 1999 for sedation of patients hospitalized in intensive care settings. Since then, a growing number of research articles have emerged reporting other possible indications such as regional and general anesthesia (12, 13). Dexmedetomidine was reported to be effective in protecting against focal ischemia in rabbits, cardiac IR injury in rats, kidney IR injury in rats, and incomplete forebrain ischemia in rats (14–17). Despite its increased clinical use, many times in critically ill patients, the effect of dexmedetomidine on liver IR injury has not been yet investigated (18).

The present study was therefore designed to evaluate the effectiveness of dexmedetomidine in an established *in vivo* rat model of hepatic ischemia/reperfusion (I/R) injury and explain the potential mechanisms involved in any beneficial effect with different parameters such as p53 related apoptosis, HSP60 expressions and oxidative stress parameters such as Thiobarbutiric Acid-Reactive Substances (TBARS) levels, glutathioneS-transferase (GST),

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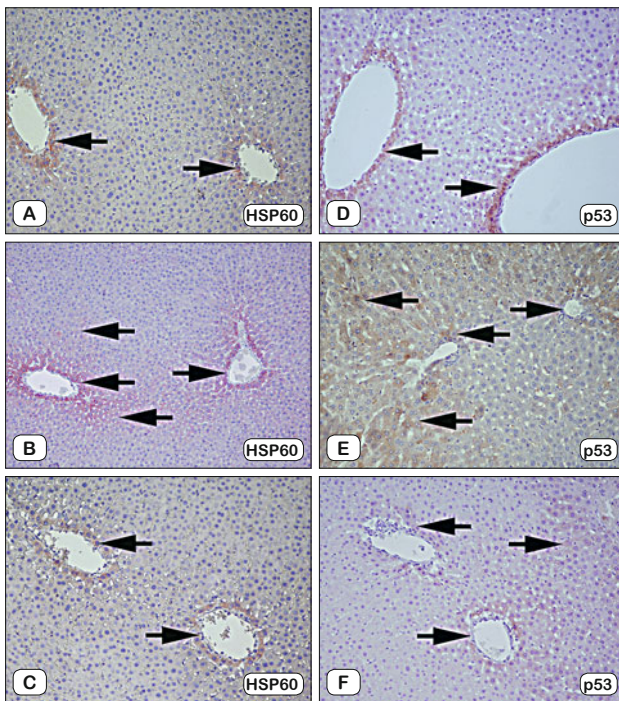
Fig. 1. Expressions of HSP60 (A to C) and p53 (D to E) in liver in all groups. Arrows: Immunopositive cells. All magnifications are x200.

superoxide dismutase (SOD), catalase (CAT), and paraoxonase 1 (PON-1) activities (Fig. 1).

Materials and methods

Animals and experimental protocol

The experiments were performed in adherence to National Institutes of Health guidelines on the use of experimental animals. Twenty-four male, Wistar rats, weighing from 275 to 350 g, were housed at constant temperature with 12/12 h periods of light and dark exposure. Animals were allowed access to standard rat chow and water *ad libitum* during an acclimation period of at least 5 days prior to use in these experiments. The approval of Institutional Ethics Committee was obtained. The experiments were performed in the Animal Experimental Laboratory of the Physiology Department in Kirikkale University.

Rats were anesthetized with intramuscular ketamine 100 mg per kg. The chest and abdomen were shaved and each animal was fixed in a supine position on the operating table. The abdomen was cleaned with 1% polyvinyl iodine and when dry, the operating field was covered with a sterile drape and median laparotomy was performed. There were three experimental groups (Group C (Control; n = 8), Group I/R (ischemia–reperfusion, n = 8), and Group I/R-D (I/R-Dexmedetomidine; n = 8). Precedex 100 μg, 2 ml (Abbott®, Abbott Laboratory, North Chicago, Illinois, USA) was administrated via intraperitoneal route 30 minutes before clampage (19).

Sham operation was performed on the rats in Group C. The sham operation consisted of mobilization of the hepatic pedicle only. The rats in this group were sacrificed 90 min after the procedure. Hepatic I/R injury was induced in Groups I/R and I/R-D, respectively by hepatic pedicle clampage using a vascular clamp as in the previous study of Yaylak et al (3). After an ischemia period of 45 min, the vascular clamp was removed. A reperfusion period was maintained for 45 min.

All the rats were given ketamine 100 mg.kg^{-1} intraperitoneally and intracardiac blood samples were obtained.

Biochemical analysis

The liver tissues were first washed with cold deionized water to discard blood contamination and then homogenized in a homogenizator (Heidolph DIAX900) at 3,000 rpm for 3 min. After centrifugation at 10,000xg for 10 min. the upper clear layer was taken. Protein and TBARS amounts were measured as described by Lowry et al. (20) and Van Ye et al (21) respectively in this fraction. Part of the homogenate was extracted in ethanol/chloroform mixture (5/3 v/v) to discard the lipid fraction, which caused interferences in the activity measurements of T-SOD, CAT and GST activities. After centrifugation at 10,000 x g for 60 min, the upper clear layer was removed and used for the T-SOD, CAT, GST analyses.

In the upper clear layer, T-SOD, CAT, GST and PON 1 enzyme activities were measured as described Durak et al (22), Aebi (23) and Habig et al (24), methods respectively. One unit of SOD activity was defined as the enzyme protein amount causing 50 % inhibition in NBTH$_2$ reduction rate, and the results were expressed in U/mg protein. The CAT activity method is based on the measurement of absorbance decrease due to H$_2$O$_2$ consumption at 240 nm. The GST activity method is based on the measurement of absorbance changes at 340 nm due to formation of GSH-CDNB complex. PON1 activity toward paraoxon was assessed by adding samples to 2 mL Tris/HCl buffer containing CaCl$_2$, paraoxon (O,O-diethyl-O-p- nitrophenylphosphate), and NaCl. The rate of generation of p-nitrophenol was determined at 405 nm and 25 °C in a spectrophotometer. Increases in absorbance were recorded at 30-sec intervals during 5 min after 30 sec of initial pre-incubation. The enzymatic activity was calculated from molar extinction coefficient (17,000 L/mol.cm).

TBARS assay was carried out using the thiobarbituric acid method and the results were given in nmol/mg protein. All the procedures were performed at 4 °C throughout the experiment.

Histopathological evaluation

All the specimens were fixed in 10 % neutral formalin and processed with classic histologic method. Several 4 μ sections obtained from these specimens were mounted on poly-L-lysine-coated slides. All the slides were immunohistochemically stained with p53 and HSP60 for detection genome destruction and tissue stress respectively.

HSP60 and p53 positive hepatocytes were counted under ImageJ Image analysis program. The immunopositive cells in 5 different areas in each slide under x20 objective magnification were counted and immunoreactivity was calculated using with HSCORE

Liver blood flow measurement

Liver blood flows were recorded. Blood flow measurements were conducted by placing and fixing the probe on the tissue by a laser Doppler microvascular perfusion monitor (OxyLab LDF; Oxford Optronix Limited, Oxford, UK).

Statistical analysis

Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 12.0 program was used for statistical analysis. Variations in liver tissue oxidative state parameters, liver blood flow, and histopathological evaluation between study groups were assessed by using Kruskal-Wallis test. Bonferroni-adjusted Mann–Whitney U test was used after significant Kruskal-Wallis to determine which group differs from the other. Results were expressed as mean ± standart deviation (Mean ± SD). Statistical significance was set at a $p < 0.05$ for all analyses.

Results

In the biochemical evaluation, all parameters, except GST levels, were significant between the groups ($p <0.05$).

SOD activities and TBARS levels were also higher in I/R group than in the other groups (SOD: Control Group- IR Group $p = 0.007$, IR Group- IRD Group $p = 0.047$; TBARS: Control Group- IR Group $p = 0.028$, IR Group- IRD Group $p = 0.011$) (Tab. 1). However, dexmedetomidine treatment in I/R-D group resulted with SOD activities and TBARS levels similar to those in the control group, (Tab. 1).

Similarly, PON-1 activity was also higher in I/R group than in other groups (Control Group- IR Group $p = 0.032$, IR Group- IRD Group $p = 0.016$) (Tab. 1).

CAT activity was also higher in Control Group than in the other groups (Control Group- IR Group $p = 0.002$, Control Group- IRD Group $p = 0.036$) (Tab. 1).
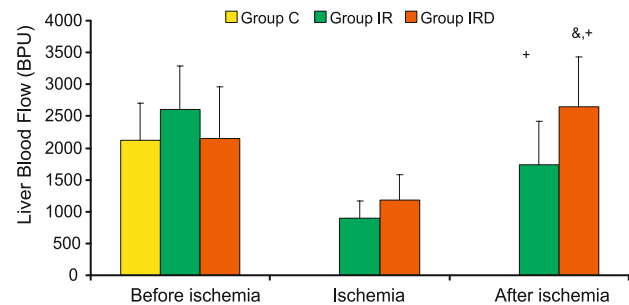
Although HSP60 expression was significantly increased among I/R, I/R-D and C groups there were no significant differences between I/R-D and C ($p = 0.443$). On the other hand, p53 expression was also significantly increased among I/R, I/R-D and C groups but there were no significant differences between I/R-D and C groups ($p = 0.354$).

In the liver blood blow measurement; it was detected that when compared with after ischemia period in Group IR, the liver blood flow in Group IRD was significantly decreased (IR Group – IRD Group $p = 0.028$) (Fig. 2). In addition, in the period after

Fig. 2. Distributions of liver blood flow measurements in blood perfusion units (BPU). $p < 0.05$ when compared to group IR.

ischemia, liver blood flow in both groups were significantly increased compared to the period of ischemia (IR Group $p = 0.017$, IRD Group $p = 0.012$) (Fig. 2).

Discussion

Hepatic I/R injury is defined as the prolongation and aggravation of ischemic tissue damage with reperfusion (26). Several cellular and molecular changes in hepatic I/R injury have been investigated in clinical and experimental studies. However, in this study, we have reported (for the first time, to our knowledge) the protective effect of dexmedetomidine on erythrocyte deformability alterations in experimental hepatic I/R injury model in the rat. Dexmedetomidine, which is a highly potent and selective α2-adrenoreceptor agonist, was administered before induction of ischemia, which resulted with decreased SOD activities and TBARS levels known to play a crucial role in the development of hepatic I/R injury.

Hepatic I/R injury induced by lipid peroxidation generates a complex variety of products. Many of these react with several protein products and DNA and cause local and systemic toxic and mutagenic effects (27). A major target site of lipid peroxidation is the cellular membrane which contains polyunsaturated fatty acids. Toxicity of partially reduced oxygen species arises from the peroxidation of polyunsaturated fatty acid of membrane phospholipids. The final event leading to structural and functional tissue damage is the loss of disintegration of cellular membrane. In hepatic I/R injury, this mechanism is exacerbated after reperfusion with microcirculatory derangements and altered mitochondrial functions (28).

On the other hand, p53 is a gene which regulates the cell cycle progression, modulates apoptosis and/or DNA repair in cellular responses to stress. While the p53 protein normally is short-lived and present at low levels in normal cells, in response to both genotoxic and nongenotoxic stresses it accumulates in the nucleus where it binds to specific DNA sequences and modulates DNA repair processes (29, 30). The arrest of cell cycle progression may provide time for the repair of DNA damage. If DNA does not repair completely, it stimulates apoptotic caspase cascades in cytoplasm and the cell kills itself. Furthermore, Heat Shock Protein 60 (HSP60) is a member of heat shock proteins which are responsible for preventing damage to proteins in response to high levels of heat. It is a mitochondrial chaperonin responsible for transportation and refolding of proteins from cytoplasm to mitochondrial matrix. Nowadays, dif-

Tab. 1. Biohemical parameters in rat liver tissues.

Groups and biochemical parameters	Group C (n = 8)	Group I/R (n = 8)	Group I/R+D (n = 8)	p
TBARS	2.13±0.90	3.55±1.30*	1.81±0.38&	0.019
GST	1.19±0.48	1.01±0.57	1.43±0.66	0.272
SOD	16.64±2.90	24.83±6.43*	17.36±6.15&	0.028
PON-1	34.45±15.9	51.52±17.2*	21.63±11.8&	0.036
CAT	5724.6±873.9	4355.9±548.2*	4383.2±419.1*	0.013

TBARS – nmol/mg protein, GST – mIU/mg protein, SOD – U/mg protein, PON-1 – mIU/mg protein, CAT – IU/mg protein, * $p < 0.05$ when compared to Group C & $p < 0.05$ when compared to Group IR

ferent studies suggest that this protein is involved in stress response, cancer, diabetes and some immunological disorders. Up-regulation of HSP60 production allows for the maintenance of different cellular processes in the cell especially during stressful time (31). In a study, it was shown that HSP's are playing an important role in the defensive mechanism against neurotoxicity elicited by free radical oxygen and nitrogen species produced in aging and neurodegenerative disorders (32). Different studies have shown that HSP60 are necessary for cellular survival under toxic and stressful circumstances (33). Four hours after reperfusion, liver transplant patients were shown to have significantly increased serum levels of HSP60 (34).

In our study, the fact that we detected that p53 was significantly upregulated in I/R group means that I/R process stimulates DNA damage. However, in I/R+D group it was shown that p53 was significantly downregulated, which means that dexmedetomidine has protective effects on I/R-related cell damage. At the same time, in I/R+D group we also detected a decrease in p53 expression/suppression profile which means that dexmedetomidine prevention has beneficial effects on cellular stress. Furthermore, the significant decrease in HSP60 expression in I/R+D group when compared to I/R group also shows the beneficial effects of dexmedetomidine on cellular stress.

Free oxygen radicals have a marked mediator role in IR injuries of several organs, including the liver (6, 35, 36). Some research suggests that antioxidant molecules may provide protection from I/R injury (6, 7, 37, 38).

In our study, by measuring the liver tissue CAT, PON-1, SOD, GST activities and TBARS levels, we obtained information about hepatic I/R. PON enzyme shows antioxidant activity by protecting LDL from oxidation and neutralizing free radicals, including hydrogen peroxide (39–41).

Oxidative damage occurs when this equilibrium is disrupted. Increased lipid peroxidation is also one of the results of increased oxidative stress (42). It was reported that some parameters of erythrocyte functions and membrane integrity were impaired in increased lipid peroxidation in *in vivo* and *in vitro* studies (43). The products that arise due to lipid peroxidation associated with increased oxidative stress significantly affect membrane permeability and micro viscosity. Thus, the diminished deformability capacity and survival of erythrocytes are observed (44). SOD, CAT, and GSH-Px are responsible for cellular antioxidant defense mechanisms. These enzymes eliminate superoxide anions and hydrogen peroxides, and prevent free radical production (45). SOD is the primary defensive enzyme against oxygen-derived free radical production and catalyses H_2O_2 conversion O_2^- (46). Oxygen radicals generated in response to I/R have been implicated in the micro vascular dysfunction and parenchymal cell injury of the intestine and liver (47, 48). The TBARS assay, which measures MDA, is a simple and easy-to-use method for assessment of the degree of injury attributable to the free radicals produced by ischemia/reperfusion, and is frequently used in laboratory (49). In this current study, increased TBARS levels were accompanied in hepatic I/R group. Dexmedetomidine treatment was also observed to be protective against increased TBARS production in hepatic I/R injury.

In this study, the decreased activity of PON, SOD, CAT activities and TBARS levels in liver tissue support the protective effect of dexmedetomidine.

In conclusion, it was found that dexmedetomidine increased the antioxidant ability and decreased oxygen free radicals in the early period of hepatic IR injury in rats.

In conclusion, the results of this study clearly demonstrated that HSP60, p53 are significantly upregulated in experimental hepatic I/R injury in the rat. The increased production of SOD, PON and TBARS, which causes lipid peroxidation and cellular membrane alterations may play an important role in alterations. However, it was observed that when given before induction of ischemia, dexmedetomidine has protective effects on these alterations in hepatic I/R injury. Other aspects of these findings including clinical significance and practical applications merit further experimental and clinical investigation.

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