

Effects of Carvedilol on liver ischemia-reperfusion injury in rats

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ABSTRACT

BACKGROUND: The aim of this study was to analyze the potential protective effect of Carvedilol against liver ischemia-reperfusion (I/R) injury in rats.

METHODS: A total of 40 Wistar albino rats were randomly divided into four groups (n=10 each). Group I (Sham/Control group) underwent only laparotomy, Group II (Carvedilol group) was administered carvedilol and then underwent laparotomy, Group III (I/R group) underwent laparotomy and hepatic ischemia/reperfusion, and Group IV (I/R + Carvedilol group) was administered carvedilol and then underwent laparotomy and hepatic ischemia/reperfusion. Blood samples were collected for malondialdehyde, glutathione (GSH), and myeloperoxidase (MPO) analysis. Liver sections were obtained for histopathological analysis and stained with hematoxylin-eosin. Tumor necrosis factor- α (TNF- α) and Caspase-3 primary antibodies were used for the immunohistochemical analysis.

RESULTS: Serum GSH levels increased in the I/R + Carvedilol group. MPO activity was increased significantly in the IR group. In I/R + Carvedilol group, serum MPO levels were similar to the control group. Histopathological findings showed reduced dilatation and congestion in vena centralis, regenerative changes in hepatocyte cells with the protected nucleus structure in the I/R + Carvedilol group. Hepatocyte nuclei with increased pycnosis and apoptosis and the dilated vena centralis were observed in I/R group. In the control group, TNF- α showed a positive reaction in macrophage cells around vena centralis. An increase in TNF- α expression was observed in hepatocyte cells of I/R group. Positive expression of caspase-3 in hepatocyte cells and a small number of endothelial and Kupffer cells were seen in I/R group. However, negative caspase-3 expression was seen in hepatocyte, endothelial, and Kupffer cells in I/R + Carvedilol group.

CONCLUSION: Carvedilol may prevent initiation of oxidative stress process, inflammation induction and apoptotic progression.

Keywords: Carvedilol; Caspase-3; ischemia-reperfusion; rat; tumor necrosis factor- α .

INTRODUCTION

Ischemia causes oxygen deficiency and disrupts cellular metabolism as a result of cessation of blood flow. Reperfusion is the restoration of blood flow and tissue damage occurs in many organs due to lack of the previous oxygen and nutrient delivery and accumulation of metabolic by-products. It constitutes many clinical complications such as ischemia/reperfusion (I/R) damage in the liver, liver resection, liver transplantation, and trauma.^[1] While vascular occlusion techniques such as Pringle maneuver and total hepatic vascular exclusion are used to

prevent excessive blood loss during liver surgery, liver transplantation involves perfusion of liver grafts with protective fluid before cold storage.^[2] In this sense, liver resection, liver transplantation, and trauma may cause ischemia reperfusion damage. A better understanding of liver I/R damage can lead to improvements in clinical care for many patients, especially those with prolonged ischemia times or marginal liver grafts for transplantation. Experimental I/R injury models provided a solid basis for the cellular and molecular mechanisms of the hepatic injury response.^[3] Carvedilol is a potent nonselective beta-blocker with mild anti-alpha 1 adrenergic activity (one-

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tenth of its beta-blocker activity). It was developed for the treatment of arterial hypertension and heart failure. It improves myocardial function, increases survival, and decreases mortality in adults with congestive heart failure.^[4] Carvedilol further has antioxidant activity, which may be of interest in patients with cirrhosis.^[5] Experimental and human studies have shown that during hypoxia, Carvedilol increases cardiomyocyte survival with the antioxidative property, inhibition of calcium antagonism, anti-arrhythmia, anti-apoptosis, and neutrophil infiltration.^[6] TNF- α is a cytokine produced by various immune cells that include macrophages/monocytes. TNF- α can induce multiple signal pathways involved in inflammation, proliferation, and apoptosis. TNF- α has long been reported as an important tool in the hepatic inflammatory response to I/R. TNF- α is released by various cells in the liver, but its release by Kupffer cells is most evident and is rapidly detected after reperfusion.^[7,8] Caspase-3 is an apoptosis marker and zymogen procaspase-3 is localized in cytosol, mitochondria and nuclei. The subcellular location of procaspase-3 in the liver has been reported to be cytosolic or mitochondrial with various studies.^[9] Various caspase substrates are involved in the regulation of DNA structure, repair, and replication. Caspase-3 substrate cleavage was observed under oxidative stress in different pathological conditions.^[10] The aim of this study was to analyze the potential protective effect of Carvedilol against liver I/R injury in rats.

MATERIALS AND METHODS

All of the procedures involved in the experimental protocols were approved by the Experimental Animal Ethics Committee of Dicle University, Diyarbakir, Turkey (Protocol No: 04.11.2020-2020/33). The experiments were performed in the University's Research and Application Center Laboratory. The study was performed in accordance with the Helsinki Declaration. The use of animals was performed within the framework of protocols in Guide for the Care and Use of Laboratory Animals released by National Research Council.^[11] Male Wistar albino rats (n=40), 34 months old, weighing 180–240 g, were used for the study and individually housed in clean cages under standard conditions (light/dark cycle of 12 h/12 h with 50–70% humidity, at 25 \pm 3°C) and fed with standard pellet diet and water ad libitum.

The animals were randomly divided into four groups of ten animal per group (n=10). Group I (Sham/Control group): Animals received no treatment and underwent midline laparotomy only; Group II (Carvedilol group): Animals underwent midline laparotomy after the administration of 30 mg/kg/day Carvedilol by oral gavage for 5 days before the operation. The last dose of Carvedilol was given 3 h before the operation; Group III (I/R group): Animals were subjected to liver ischemia and reperfusion after administration of vehicle (distilled water) for 5 days by gavage before the induction of hepatic ischemia; and Group IV (I/R + Carvedilol): Animals underwent liver ischemia and reperfusion after the administration

of 30 mg/kg/day Carvedilol by gavage for 5 days before the induction of hepatic ischemia. The last dose of Carvedilol was given 3 h before the operation.

Surgical Procedure

Anesthesia was provided using 50 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun; Bayer AG, Leverkusen, Germany) through intramuscular injection and the experimental procedure was initiated. Until the end of the experiment, anesthesia was given at the same dose if required again. No additional drugs for euthanasia or analgesia were used. The rats were anesthetized throughout the experiment and were sacrificed by exsanguination at the end of the experiment. Furthermore, no antibiotics were used during the experiment. All subsequent procedures were performed using aseptic technique with sterile equipment. Initially, abdominal hair was removed, and skin was prepared with a Povidone-iodine solution (Betadine). Afterward, the rats underwent a 4 cm midline abdominal incision through the musculature and peritoneum. The hepatoduodenal ligament was observed. 3 h after Carvedilol administration, ischemia and reperfusion period (each lasted for 30 min) of the liver with immediate occlusion of the hepatoduodenal ligament was carried out. T_{max} (time to peak drug concentration) of Carvedilol in rats after oral administration is 2.8 h.^[12] Hence, at the time of reperfusion, the drug concentration was highest. In the I/R and the I/R + Carvedilol groups, the portal vein and hepatic artery were occluded with the use of a rubber band and silk suture, and the period of ischemia was initiated. After a 30 min ischemic period, the suture was loosened, and 30 min of reperfusion period began. During these periods, the abdomen was closed at the midline. At the end of the reperfusion period, the abdomen was reopened, and blood samples were collected from the heart.

Biochemical Analysis

Blood samples were collected in tubes with a gel separator and centrifuged for 5 min at 1550 g. The supernatant plasma was removed and placed in polypropylene plastic tubes. The tubes were properly labeled with the appropriate sample name and type. Samples were taken and stored at -80°C for the determination of the MDA, GSH, and MPO. The MDA levels were estimated using the double-heating method of Draper et al.^[13] This method is based on spectrophotometric measurements of the color generated by the reaction of thiobarbituric acid and MDA.

Histopathological Analysis

Liver sections were obtained for histopathological analysis and were fixed in 10% buffered formalin, dehydrated in ethanol (50–100%), purified in xylene, and embedded in paraffin. Sections (4–5 mm thick) were cut and stained with hematoxylin and eosin (H-E). The sections were studied to assess the pathological changes in the liver. The severity of

hepatic injury was assessed using an ordinal grading system as follows:

Grade 0: Minimal or no sign of injury; Grade 1: Mild injury accompanied by cytoplasmic vacuolation and focal nuclear pyknosis; Grade 2: Moderate injury with cytoplasmic vacuolization, confluent areas of hepatocyte ballooning with no obvious necrosis, sinusoidal dilatation and congestion, and loss of intercellular borders; Grade 3: Moderate to severe injury with areas of coagulative necrosis, widespread sinusoidal dilatation, and congestion; and Grade 4: Severe injury with severe confluent coagulative necrosis, and disintegration of and hemorrhage into hepatic chords leading to the loss of tissue architecture.^[14]

Immunohistochemical Analysis

Formaldehyde-fixed tissue was embedded in paraffin wax for further immunohistochemical examination. Sections were deparaffinized in xylene and passed through descending alcohol series. The antigen retrieval process was performed in citrate buffer solution (pH=6.0) for 15 min in a microwave oven at 700 W. Sections were allowed to cool at room temperature for 30 min and washed twice in phosphate buffered saline (PBS) for 5 min. Endogenous peroxidase blockage was performed in a 3% hydrogen peroxide solution for 7 min. The washed samples were incubated in Ultra V block (catalog no. TA-015UB, Thermo Fischer, US) for 8 min. Blocking solution was removed from the sections and allowed to incubate overnight at +4 ° C with primary antibodies TNF- α , (catalog no: ab220210, Abcam, US) and caspase-3 (catalog no: ab208161, Abcam, US). After washing the sections in PBS, secondary antibody (TP-015-BN, Thermo Fischer, US) was applied for 20 min. The sections were washed in PBS for 2 \times 5 min and then exposed to streptavidin-peroxidase (TS-015-HR, Thermo Fischer, US) for 20 min. Sections washed with PBS were allowed to react with DAB (TA-001-HCX, Thermo Fischer, US) chromogen. Counterstaining with hematoxylin was applied and after washing, the preparations were mounted. Sections were examined under a light microscope (Zeiss Imager A2, Germany).

Statistical Analysis

The data obtained in the study were expressed as arithmetic mean \pm standard deviation. Statistical analyzes were made using the SPSS 22 (SPSS Inc. Chicago, IL, USA) program. Kruskal–Wallis test and Dunn-Bonferroni post hoc tests were used in comparison of the groups. $P < 0.05$ was taken as the significance level. TNF- α and caspase-3 expression levels were statistically analyzed with IBM SPSS Statistics 25 program. Shapiro–Wilk test ($p < 0.05$) was used to check the distribution of data. The means of the groups were compared using the Kruskal–Wallis non-parametric test and significance was recorded between the means ($p = 0.01$). In comparison between groups, one-way ANOVA, *post hoc* test was performed with Tamhane's T2 test. Results are shown as mean + standard deviation (SD).

RESULTS

Evaluation of Histopathological Scores in the Liver and Biochemical (MDA, GSH, and MPO) Results

There were statistically significant differences between the groups in terms of histopathological scores. The liver histopathological scores were significantly higher in ischemia-reperfusion (I/R) group and I/R + Carvedilol group than the sham group. The mean histopathological score of I/R + Carvedilol group was significantly lower than the I/R group. The histopathological scores of the liver tissues of the groups are shown in Table 1.

The MDA, GSH, and MPO values of the control group and the Carvedilol group were compared among themselves. There was no significant difference between these groups. When the control and Carvedilol groups were compared with the I/R group, the lipid peroxidation index MDA was higher in I/R group. Serum MDA levels were decreased in the I/R + Carvedilol group and were close to the values of the control and Carvedilol groups. After ischemia-reperfusion, the level of GSH in blood tissue decreased significantly in I/R group compared to the control group. Serum GSH levels started to increase in the I/R + Carvedilol group. MPO activity was increased significantly in the I/R group. In the I/R + Carvedilol group, serum MPO levels were similar to the control group, while Carvedilol provided protection in terms of neutrophil infiltration. Measurements of biochemical parameters and histopathological scores were shown in Table 1. Graphical representation of histopathological results is shown in Figure 1.

As a result of ischemia reperfusion, it was observed that increased lipid peroxidation in the liver and decreased GSH and increased MPO levels caused oxidative tissue damage, while carvedilol treatment protected the liver against oxidative damage (Table 1 and Fig. 2).

Histopathological Finding

Control group: In the liver parenchyma, polygonal-shaped chromatin-rich hepatocyte cells were observed in radial organization around vena centralis with a small thin lumen structure close to the middle part. Between the hepatocytes, endothelial cells of liver sinusoids and triangular Kupffer cells were observed in the periphery (Fig. 3a). Carvedilol group: Mild dilatation in the sinusoidal area and irregularity between the cells was observed. There was no inflammatory cell infiltration (Fig. 3b). I/R group: Increased pyknotic and apoptotic nuclei were abundant in hepatocyte cell and vena centralis was dilated. An increase in inflammatory cells in the liver portal area and periportal area, congestion in blood vessels, degeneration in bile duct cells in the interlobular area were observed (Fig. 3c). I/R + Carvedilol: Decreased dilatation and congestion in vena centralis was observed. Hepatocyte cells

Table 1. Measurements of histopathological scores and biochemical parameters

Parameter	Groups (n=10)	Mean±SD	Mean Rank	Kruskal-Wallis Test value	Multiple comparisons for groups (Dunn-Bonferroni test) (p<0.05)
Degeneration in hepatocyte cells	(1) Control	0.50±0.52	7.75	31.550 p=0	(3)(4)
	(2) Carvedilol	0.54±0.45	7.40		(3)(4)
	(3) Ischemia/Reperfusion	3.40±0.51	31.00		(1)(2)(4)
	(4) Ischemia/Reperfusion +Carvedilol	1.20±0.63	13.25		(1)(2)(3)
Vascular dilatation and congestion	(1) Control	0.50±0.52	7.25	30.419 p=0	(3)(4)
	(2) Carvedilol	0.47±0.48	6.15		(3)(4)
	(3) Ischemia/Reperfusion	3.20±0.63	30.00		(1)(2)(4)
	(4) Ischemia/Reperfusion +Carvedilol	1.30±0.48	13.90		(1)(2)(3)
Inflammation	(1) Control	0.40±0.51	7.20	32.081 p=0	(3)(4)
	(2) Carvedilol	0.53±0.44	7.90		(3)(4)
	(3) Ischemia/Reperfusion	3.80±0.42	31.50		(1)(2)(4)
	(4) Ischemia/Reperfusion +Carvedilol	1.30±0.67	13.80		(1)(2)(3)
MDA (nmol/g)	(1) Control	32.10±3.69	7.60	29.943 p=0	(3)(4)
	(2) Carvedilol	37.20±3.70	28.70		(3)(4)
	(3) Ischemia/Reperfusion	55.20±4.05	32.30		(1)(2)(4)
	(4) Ischemia/Reperfusion +Carvedilol	36.12±3.03	13.40		(1)(2)(3)
MPO (U/g)	(1) Control	3.62±0.42	10.20	29.758 p=0	(3)
	(2) Carvedilol	3.54±0.52	28.40		(3)
	(3) Ischemia/Reperfusion	7.69±0.69	32.60		(1)(2)(4)
	(4) Ischemia/Reperfusion +Carvedilol	3.69±0.27	10.80		(1)(2)(3)
GSH (µmol/g)	(1) Control	3.62±0.42	35.50	33.493 p=0	(3)(4)
	(2) Carvedilol	3.66±0.48	7.05		(3)(4)
	(3) Ischemia/Reperfusion	0.67±0.08	13.95		(1)(2)(4)
	(4) Ischemia/Reperfusion +Carvedilol	1.05±0.07	25.50		(1)(2)(3)

MDA: Malondialdehyde; MPO: Myeloperoxidase; GSH: Glutathione; SD: Standard deviation.

with the protected nucleus structure showed regenerative changes in the I/R + Carvedilol group. While dilatation continued in the sinusoidal area, a small number of inflammatory cell infiltrations was observed in the peri portal area (Fig. 3d).

Immunohistochemical Findings

TNF- α Expression

Control group: TNF- α showed a positive reaction in macrophage cells around vena centralis and small blood vessels

in the periportal area and Kupffer cells in the periphery of the sinusoidal area. TNF- α expression was negative in the majority of the lobules (Fig. 4a). Carvedilol group: TNF- α reaction was observed in some of degenerated hepatocyte cells and Kupffer cells, however many of hepatocyte and Kupffer cells showed negative TNF- α expression (Fig. 4b). I/R group: Throughout the liver lobules, an increase in TNF- α expression was observed in hepatocyte cells which underwent apoptotic period, endothelial and Kupffer cells and con-

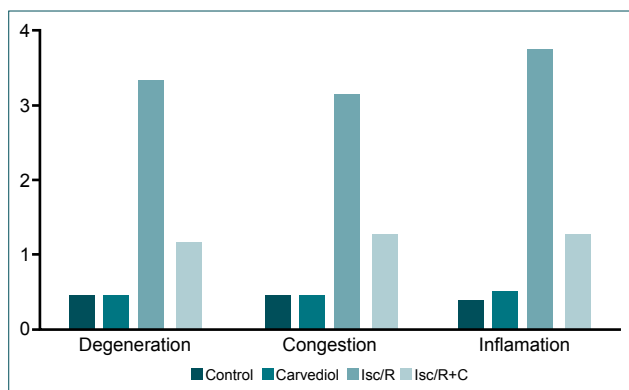


Figure 1. Graphical representation of histopathological results.

nective tissue cells in the portal and peri-portal space (Fig. 4c). I/R + Carvedilol: TNF- α expression was moderate in some hepatocyte cells and a few endothelial and Kupffer cells around vena centralis; however, it was negative in connective tissue cells in the periportal area (Fig. 4d).

Caspase-3 Expression

Control group: In the liver lobule around vena centralis and toward the periportal area, Caspase-3 showed a positive reaction in a small number of hepatocyte cells, but it was negative in the majority of the lobule, hepatocyte, endothelial, and Kupffer cells (Fig. 5a). **Carvedilol group:** Caspase-3 reaction was positive in some of hepatocyte, endothelial, and Kupffer cells but it was mostly negative in those cells within the liver lobule (Fig. 5b). **I/R group:** Hepatocytes, endothelial and Kupffer cells, as well as connective tissue cells in the portal and peri-portal space and cells in the bile duct showed Caspase-3 positive reaction (Fig. 5c). **I/R + Carvedilol:** Positive expression of Caspase-3 was seen in some hepatocyte cells around vena centralis and a few solitary hepatocyte cells in the lobule and a small number of endothelial and Kupffer cells (Fig. 5d).

Statistical comparisons of TNF- α and Caspase-3 expression between groups and mean \pm SD values are shown in Table 2.

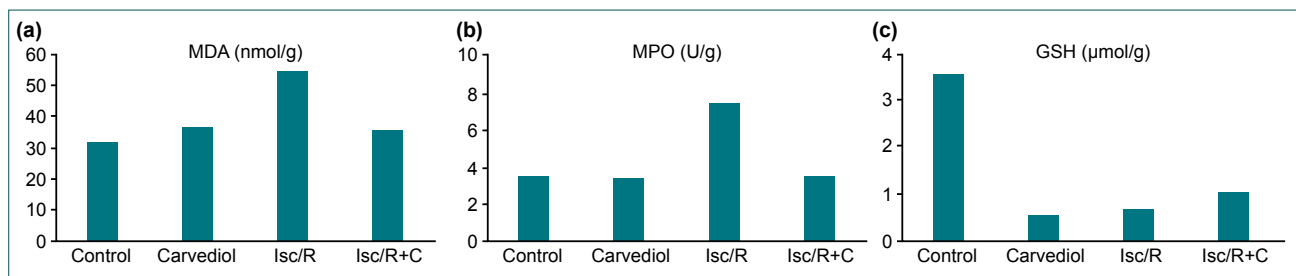


Figure 2. (a) Graphical representation of MDA values. (b) Graphical representation of MPO values. (c) Graphical representation of GSH values.

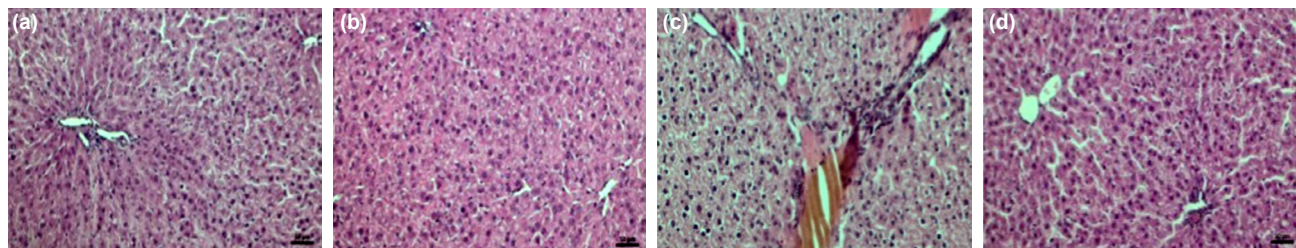


Figure 3. (a) Control group: Normal appearance of the liver lobule, Hematoxylin-Eosin staining Bar 50 μ m, (b) Carvedilol group: Dense chromatin in hepatocyte cells, slight dilatation in the sinusoidal area, no inflammation, Bar 50 μ m, (c) I/R: Increased apoptosis in hepatocyte cells, an increase in inflammatory cells in the portal area and periportal area, congestion in blood vessels, Hematoxylin-Eosin staining Bar 50 μ m, (d) I/R+Carvedilol: A decrease in dilatation and congestion in the vena centralis, regenerative changes in hepatocyte cells, decrease in inflammatory cell infiltration, Hematoxylin-Eosin staining Bar 50 μ m.

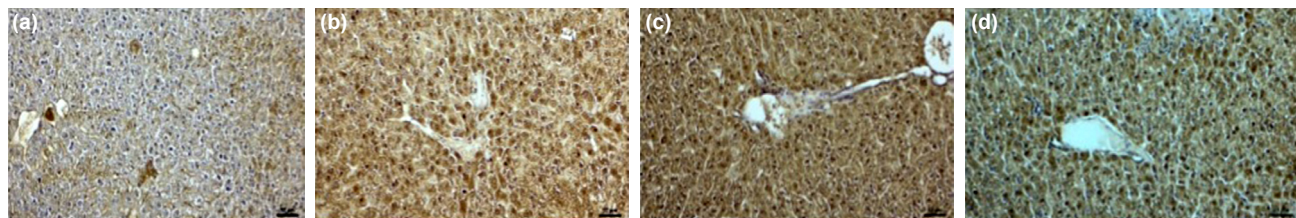


Figure 4. (a) Control group: Positive TNF- α reaction in a small number of macrophage cells and Kupffer cells in the periphery of the sinusoidal area, TNF- α immunostaining Bar 50 μ m, (b) Carvedilol group: Positive TNF- α expression in some degenerated hepatocytes cells, endothelial cell and Kupffer cells, TNF- α immunostaining, Bar 50 μ m, (c) I/R group: A significant increase in the expression of TNF- α in hepatocyte cells, endothelial and Kupffer cells, inflammatory cells in the portal and peri-portal space, TNF- α immunostaining, Bar 50 μ m, (d) I/R+Carvedilol: Mild TNF- α expression in hepatocyte cells, endothelial and Kupffer cells, TNF- α immunostaining, and Bar 50 μ m.

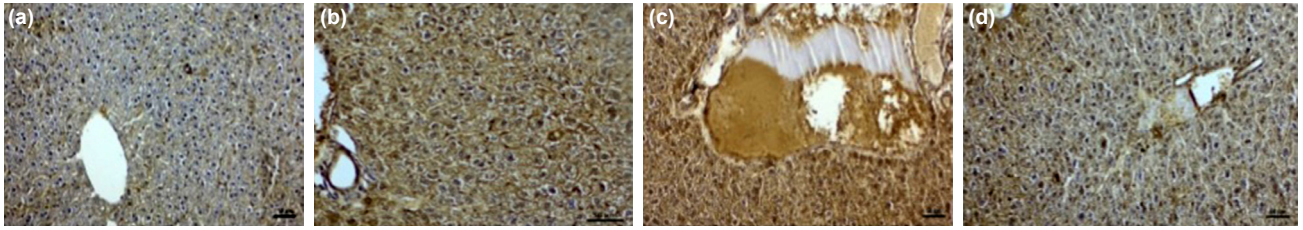


Figure 5. (a) Control group: Negative Caspase-3 expression in hepatocyte, endothelium, Kupffer cells, Caspase-3 immunostaining, Bar 50 μ m, (b) Carvedilol group: Positive Caspase-3 expression in some degenerative hepatocyte cells, Kupffer cells, Caspase-3 immunostaining, Bar 50 μ m, (c) I/R group: Positive Caspase-3 expression in hepatocyte cells, endothelial and Kupffer cells, as well as connective tissue cells in the portal and peri-portal space and bile duct cells, Caspase-3 immunostaining Bar 50 μ m, (d) I/R+Carvedilol: Positive expression of Caspas-3 in hepatocyte cells and a small number of endothelial and Kupffer cells, Caspase-3 immunostaining Bar 50 μ m.

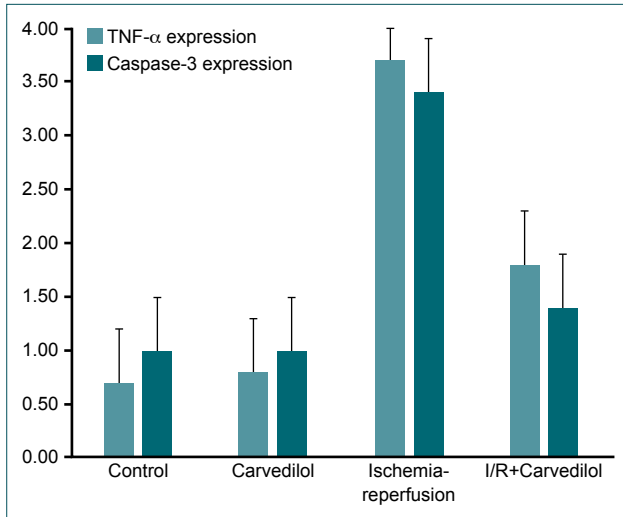


Figure 6. Averages of TNF- α and Caspase-3 expressions by groups.

According to the statistical analysis, no significant difference was observed between the control group and the Carvedilol group in terms of TNF- α expression ($p=1.00$). TNF- α expression was higher in the I/R group and these values were statistically significant compared to the control ($p=0.01$) and

Carvedilol ($p=0.01$) groups ($p=0.01$). TNF- α expression was significantly lower in I/R + Carvedilol group compared to I/R group ($p=0.01$).

No significance was observed between the Control group and the Carvedilol group in terms of caspase-3 expression level ($p=0.01$). The caspase-3 expression level was the highest in the I/R group, and the difference was significant for both groups when compared with the control ($p=0.01$) and Carvedilol ($p=0.01$) groups. In the I/R + Carvedilol group, the caspase-3 expression level was decreased compared to the I/R group, and this decrease was statistically significant ($p=0.01$). The values in Table 2 are shown in Figure 6.

DISCUSSION

During liver ischemia, oxygen deficiency in hepatocytes due to depletion of ATP in cell damage causes swelling of mitochondrial degeneration in sinusoidal endothelial cells and Kupffer cells. Activation of Kupffer cells with the production of reactive oxygen species results in upregulation of pro-inflammatory cytokines, neutrophil-mediated injury. These are the main factors contributing to inflammation-related damage.^[15] In other models of severe liver damage, when hepato-

Table 2. Mean of TNF- α and Caspase-3 expression by groups and comparison between groups

Parameter	Groups	n	Mean \pm SD	Mean Rank	Kruskal-Wallis Test value	Multiple comparisons for groups ($p<0.05$)
TNF- α expression	(1) Control	10	0.70 \pm 0.67	11.80	27.390 $p=0.01$	(3)(4)
	(2) Carvedilol	10	0.80 \pm 0.79	12.80		(3)
	(3) Ischemia/Reperfusion (I/R)	10	3.70 \pm 0.48	35.20		(1)(2)(4)
	(4) I/R + Carvedilol	10	1.80 \pm 0.79	22.20		(1)(3)
Caspase-3 expression	(1) Control	10	1.00 \pm 0.67	14.00	24.569 $p=0.01$	(3)
	(2) Carvedilol	10	1.00 \pm 0.67	14.00		(3)
	(3) Ischemia/Reperfusion (I/R)	10	3.40 \pm 0.70	35.10		(1)(2)(4)
	(4) I/R + Carvedilol	10	1.40 \pm 0.52	18.90		(3)

TNF- α : Tumor necrosis factor- α ; SD: Standard deviation.

cyte loss is excess and parenchymal proliferative capacity is impaired, activation of hepatic progenitor cells occurs simultaneously with the reaction in the canal system. The expansion of hepatic progenitor cells after liver damage is associated with the severity of hepatocyte loss.^[16] It has been stated that vacuolization and sinusoidal congestion are indicators of hepatocellular damage and can be reversible, and there is no reversal of necrosis and apoptosis.^[17] In our study, due to the effect of carvedilol, a slight widening in the sinusoidal area and irregularity between the cells was observed, but there was no increase in inflammatory cell infiltration (Fig. 3b). During the I/R phase, an increase in cells with apoptotic changes was observed leading to increase in cell degeneration and inflammation (Fig. 3c). In the I/R + Carvedilol group, dilation in the sinusoidal area and reduction in inflammation and degenerative cells significantly affected apoptosis and led to a pronounced regeneration in hepatocyte cells (Fig. 3d).

Malondialdehyde (MDA) is a determinant of lipid peroxidation and provides the emergence of free radical. It can trigger various defense mechanisms with tissue damage caused by the production of reactive oxygen species. The primary defense mechanism is superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH). GSH and SOD are the most important endogenous antioxidant defense mechanisms against oxidative stress.^[18]

Ischemia-reperfusion causes a significant increase in MDA level, which is an important marker in determining lipid peroxidation. It has been observed that Carvedilol treatment eliminates this increase in MDA by removing hydroxyl and peroxy radicals and reduces lipid peroxidation. Glutathione is among the important components of intracellular protective mechanisms against a variety of harmful stimuli, including oxidative stress. It has been reported that depletion of tissue GSH, as observed in liver damage caused by ischemia and I/R, is one of the important factors that cause lipid peroxidation as well as tissue damage.^[19] The function of Carvedilol as a free radical scavenger can be interpreted as ischemia and ischemia-reperfusion increased lipid peroxidation to increase available free GSH, which detoxifies reactive intermediate oxygen products.

MPO activity is an enzyme that explains the presence of neutrophils, and the presence of high MPO activity in the liver explains the filtration of increased neutrophil, damage induced by ischemia and ischemia-reperfusion. In the ischemia reperfusion group of our study, it was observed that an increased MPO level developed with increased lipid peroxidation and decreased GSH in the liver, resulting in oxidative tissue damage. Carvedilol treatment in ischemia-reperfusion was observed to be close to the values in the control group and only Carvedilol treated group. Therefore, it was thought to protect the liver against oxidative damage (Table 1).

It is reported that ischemia increases the cytosolic calcium concentration, which, in turn, increases phospholipase A2

and the activity of lipoxygenase to form leukotrienes.^[20] Consequently, reduced blood flow or hypoxia, which alters mitochondrial respiratory chain function, reduces the redox state of mitochondrial enzymes. This reduction was predicted to result in inhibition of the oxidative phosphorylation process.

Liver I/R damage occurs with many clinical conditions such as liver bleeding and shock, surgical resection, and transplantation. Although the pathophysiology of I/R damage involves multiple signal path fluxes, inflammatory cells and soluble factors play a key role. TNF- α is implicated as the primary mediator of inflammation during I/R injury in several tissues, including the lung, heart, liver, eye, kidney, and brain. TNF- α is a potent pro-inflammatory cytokine that targets various cell types through receptor-mediated signal transduction pathways.^[21] TNF- α can induce multiple signal pathways involved in inflammation, proliferation and apoptosis. TNF- α has long been reported as an important tool in the hepatic inflammatory response to I/R. TNF- α is released by various cells in the liver, but its release by Kupffer cells is most evident and is rapidly detected after reperfusion.^[7,8] In one study, in Kupffer cells isolated from rat liver, the increase in TNF- α levels after I/R was 5 times higher than the increase in TNF- α levels in the control group.^[21] In our study, TNF- α expression was found to be positive in hepatocytes, Kupffer, and endothelial cells due to increased inflammation in the I/R stage [Figure 4c]. The increase in TNF- α expression due to increased inflammation in the I/R phase was considered as a sign of the proapoptotic induction signal. TNF- α expression due to decreased liver inflammation in rats treated with Carvedilol was moderately detected in some hepatocyte cells and a few endothelial and Kupffer cells around vena centralis; however, it was thought that it may be a sign of the beginning of cell regeneration in the liver (Fig. 4d).

Caspase-3 is an apoptosis marker where zymogen procaspase-3 is localized in cytosol, mitochondria and nuclei. The subcellular location of procaspase-3 in the liver has been reported to be cytosolic or cytosolic and mitochondrial with various studies.^[9] Various caspase substrates are involved in the regulation of DNA structure, repair, and replication. Caspase-3 substrate cleavage was observed under oxidative stress in different pathological conditions.^[10]

Proapoptotic proteins, protease caspase-8 and caspase-3, are activated during reperfusion. They cause cytochrome-c to be released into the cytoplasm along with changes in the mitochondrial membrane within the cell. These events eventually lead to the destruction of nuclear DNA and cell death.^[22] In some studies, it has been shown that ischemia induces cell destruction and apoptosis with decreased Bcl-2 levels and increased caspase 3 levels in the ischemic nucleus.^[23-25] Apoptosis levels in hepatocytes indicate changes in liver function and reserve capacity. In our study of I/R application, a significant increase in caspase-3 expression was observed in hepatocyte cells in the liver lobule, Kupffer cells and in connective tissue

cells in the periportal area (Fig. 5c). In I/R + Carvedilol group; caspase-3 expression was observed in some Kupffer cells and in a small number of hepatocyte cells in the liver lobule; however, it was mostly negative through the liver due to the Carvedilol regenerative effect (Fig. 5d).

Conclusion

Ischemia and reperfusion are characterized by the increase of neutrophils in response to free oxygen radical formation and tissue damage. It has been observed that the TNF- α co-increasing signal activity induces cell apoptosis and consequently the caspase-3 activity increases. Carvedilol, which has anti-inflammatory properties, has been shown to regulate neutrophil migration and activation, and tend to slow down inflammation and slow apoptosis by suppressing molecules that mediate inflammation. It is thought that Carvedilol may prevent initiation of oxidative stress process, inflammation induction, and apoptotic progression.

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REFERENCES

- Huguet C, Gavelli A, Bona S. Hepatic resection with ischemia of the liver exceeding one hour. *J Am Coll Surg* 1994;178:454–8.
- Lemasters JJ, Thurman RG. Reperfusion injury after liver preservation for transplantation. *Annu Rev Pharmacol Toxicol* 1997;37:327–8.
- Konishi T, Lentsch AB. Hepatic ischemia/reperfusion: Mechanisms of tissue injury, repair, and regeneration. *Gene Expr* 2017;17:277–87.
- Granger DN. Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *Am J Physiol* 1988;255:H1269–75. [CrossRef]
- Bosch J. Carvedilol for portal hypertension in patients with cirrhosis. *Hepatology* 2010;51:2214–8. [CrossRef]
- Hu H, Li X, Ren D, Tan Y, Chen J, Yang L, et al. The cardioprotective effects of carvedilol on ischemia and reperfusion injury by AMPK signaling pathway. *Biomed Pharmacother* 2019;117:109106. [CrossRef]
- Inokuchi S, Aoyama T, Miura K, Osterreicher CH, Kodama Y, Miyai K, et al. Disruption of TAK1 in hepatocytes causes hepatic injury, inflammation, fibrosis, and carcinogenesis. *Proc Natl Acad Sci U S A* 2010;107:844–9. [CrossRef]
- Yang L, Inokuchi S, Roh YS, Song J, Loomba R, Park EJ, et al. Transforming growth factor- β signaling in hepatocytes promotes hepatic fibrosis and carcinogenesis in mice with hepatocyte-specific deletion of TAK1. *Gastroenterology* 2013;144:1042–54.e4. [CrossRef]
- Miliasav I, Nipic D, Suput D. The riddle of mitochondrial caspase-3 from liver. *Apoptosis* 2009;14:1070–5. [CrossRef]
- Nicholson DW, Thornberry NA. Caspases: Killer proteases. *Trends Biochem Sci* 1997;22:299–306.
- Council NR. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press; 2011. p. 246.
- Beddies G, Fox PR, Papich MD, Kanikanti VR, Krebber R, Keene BW. Comparison of the pharmacokinetic properties of bisoprolol and carvedilol in healthy dogs. *Am J Vet Res* 2008;69:1659–63. [CrossRef]
- Draper HH, Csallany AS, Hadley M. Urinary aldehydes as indicators of lipid peroxidation in vivo. *Free Radic Biol Med* 2000;29:1071–7.
- Knudsen AR, Kannerup AS, Grønbaek H, Dutoit SH, Nyengaard JR, Funch-Jensen P, et al. Quantitative histological assessment of hepatic ischemia-reperfusion injuries following ischemic pre- and post-conditioning in the rat liver. *J Surg Res* 2013;180:e11–20. [CrossRef]
- Kim YI. Ischemia-reperfusion injury of the human liver during hepatic resection. *J Hepatobiliary Pancreat Surg* 2003;10:195–9. [CrossRef]
- Katoonzadeh A, Nevens F, Verslype C, Pirenne J, Roskams T. Liver regeneration in acute severe liver impairment: A clinicopathological correlation study. *Liver Int* 2006;26:1225–33. [CrossRef]
- Li T, Ke W, Sun P, Chen X, Belgaumkar A, Huang Y, et al. Carvedilol for portal hypertension in cirrhosis: Systematic review with meta-analysis. *BMJ Open* 2016;6:e010902. [CrossRef]
- Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. *Cardiovasc Res* 2004;61:461–70. [CrossRef]
- Shehirli O, Ozel Y, Dulundu E, Topaloglu U, Ercan F, Sener G. Grape seed extract treatment reduces hepatic ischemia-reperfusion injury in rats. *Phytother Res* 2008;22:43–8. [CrossRef]
- Shuh M, Bohorquez H, Loss GE Jr., Cohen AJ. Tumor necrosis factor- α : Life and death of hepatocytes during liver ischemia/reperfusion injury. *Ochsner J* 2013;13:119–30.
- Atalay S, Soyulu B, Aykaç A, Ögünç AV, Çetinel Ş, Özkan N, et al. Protective effects of St. John's wort in the hepatic ischemia/reperfusion injury in rats. *Turk J Surg* 2018;34:198–204. [CrossRef]
- Jaesche H. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G15–26. [CrossRef]
- Guan J, Li H, Lv T, Chen D, Yuan Y, Qu S. Bone morphogenetic protein-7 (BMP-7) mediates ischemic preconditioning-induced ischemic tolerance via attenuating apoptosis in rat brain. *Biochem Biophys Res Commun* 2013;441:560–6. [CrossRef]
- D'Amelio M, Cavallucci V, Ceconci F. Neuronal caspase-3 signaling: Not only cell death. *Cell Death Differ* 2010;17:1104–14. [CrossRef]
- Cory S, Adams JM. The Bcl2 family: Regulators of the cellular life-or-death switch. *Nat Rev Cancer* 2002;2:647–56. [CrossRef]

DENEYSEL ÇALIŞMA - ÖZ

Karvedilol'ün sıçanlarda karaciğer iskemi-reperfüzyon hasarı üzerine etkileri

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AMAÇ: Bu çalışmanın amacı, karvedilol'ün sıçanlarda karaciğer iskemi-reperfüzyon (I/R) hasarına karşı potansiyel koruyucu etkisini analiz etmektir.

GEREÇ VE YÖNTEM: Toplam 40 Wistar albino sıçan rastgele dört gruba ayrıldı (her biri n=10). Grup I'e (Sham/Kontrol grubu) sadece laparotomi, Grup II'ye (Karvedilol grubu) karvedilol uygulandı ve ardından laparotomi yapıldı, Grup III'e (I/R grubu) laparotomi uygulandı ve hepatik iskemi/reperfüzyon yapıldı ve Grup IV'e (I/R+Karvedilol grubuna) karvedilol uygulandı ve ardından laparotomi ve hepatik iskemi/reperfüzyon yapıldı. Malondialdehide (MDA), glutatyon (GSH) and miyeloperoksidaz (MPO) analizi için kan örnekleri toplandı. Histopatolojik analiz için karaciğer kesitleri alındı ve hematoksilin-eozin ile boyandı. İmmünohistokimyasal analiz için tümör nekroz faktör- α (TNF- α) ve kaspaz-3 primer antikorları kullanıldı.

BULGULAR: Serum GSH seviyeleri I/R+Karvedilol grubunda arttı. I/R grubunda MPO aktivitesi önemli ölçüde artmıştır. I/R+Karvedilol grubunda serum MPO seviyeleri kontrol grubuna benzerdi. Histopatolojik bulgular, vena centralis'te azalmış dilatasyon ve tıkanıklık, I/R+Karvedilol grubunda korunmuş çekirdek yapısı ile hepatosit hücrelerinde rejeneratif değişiklikler gösterdi. I/R grubunda artmış piknoz ve apoptozlu hepatosit çekirdekleri ve dilate vena centralis izlendi. Kontrol grubunda TNF- α , vena centralis çevresindeki makrofaj hücrelerinde pozitif reaksiyon gösterdi. I/R grubu hepatosit hücrelerinde TNF- α ekspresyonunda artış gözlemlendi. Hepatosit hücrelerinde Kaspaz-3'ün pozitif ekspresyonu ve I/R grubunda az sayıda endotel ve Kupffer hücre görüldü. Ancak I/R+Karvedilol grubunda hepatosit, endotelial, kupffer hücrelerinde negatif Kaspaz-3 ekspresyonu görüldü.

TARTIŞMA: Karvedilol oksidatif stres sürecinin başlamasını, enflamasyon indüksiyonunu ve apoptotik ilerlemeyi önleyebilir.

Anahtar sözcükler: İskemi-reperfüzyon; Karvedilol; Kaspaz-3; sıçan; TNF- α .

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