The effects of a Cilostazol, a selective phosphodiesterase III inhibitor, on liver ischemic-reperfusion injury and liver regeneration; In vitro experimental study

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Research Article

Keywords: Cilostazol, ischemic-reperfusion injury, liver regeneration

Posted Date: June 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1705397/v1

Abstract

Introduction

Hepatectomy and transplantation cause liver damage through ischemic reperfusion and oxidative stress. There is no treatment available to improve liver regeneration and reduce ischemic-reperfusion injury. The present study aimed to investigate whether a selective phosphodiesterase III inhibitor, Cilostazol, improves ischemic reperfusion injury and liver regeneration following extended hepatectomy.

Material and Method

Wistar albino rats (n=40) were randomized and divided into four equal groups. All rats underwent 60% hepatectomy, and Cilostazol (5 mg/kg per day) was administered to the experimental group. The subjects were sacrificed on the 4th and 7th days following the resection. Blood samples were taken to evaluate liver enzymes (ALT, AST), and liver tissue samples were taken to analyze morphology. Biochemical, morphological, and histopathological parameters were compared between Groups.

Results

No statistically significant differences were detected in ALT, AST values , and relative liver weights in rats treated with Cilostazol compared to the control Group without Cilostazol. Although not statistically significant, a significant increase was detected in relative liver weight and a decrease in AST value in rats treated with Cilostazol. SOD activity was significantly higher, and GSH levels, MPO, and AOPPs were significantly lower in Cilostazol applied Groups. It is seen in these findings that selective inhibition of PDE3 by Cilostazol improves hepatic circulation. It was also found that ischemic reperfusion injury decreased, and regeneration markers such as mitosis index, even nucleus, and proliferating cell nuclear antigen ratio increased in rats treated with Cilostazol.

Conclusion

The present study found that selective PDE3 inhibitor Cilostazol positively affected the histopathological parameters following extended liver resection and significantly increased hepatocellular proliferation.

Introduction

Liver damage can occur due to many different mechanisms through hepatectomy, transplantation, hypovolemia, cardiogenic shock cause liver damage, Ischemic-Reperfusion Injury (IRI), and oxidative stress (1). Both major hepatectomy and liver transplantation result in significant hemodynamic changes. The inhibition of apoptosis, acute inflammatory process, oxidant damage, and providing oxygenation following hepatectomy and liver transplantation seem to be effective methods to protect the liver against IRI (2–3). Animal model studies showed that drip infusion of phosphodiesterase-3 (PDE3) inhibitors reduces hepatic IRI (4).

Cilostazol, which is a selective PDE3 inhibitor, is a type III phosphodiesterase (PDE3) inhibitor with partial type V phosphodiesterase activity and was administered to increase the level of cyclic adenosine monophosphate (cAMP) (5). PDE3 inhibitors have anti-platelet aggregation effects by inhibiting platelet function (6). It is also known that PDE3 inhibitors increase Nitric Oxide (NO) release from endothelial cells and cause vasodilation (7). It was also reported that PDE3 inhibitors benefit hepatic IRI with increased blood flow to hepatic tissues, platelet anti-aggregation effects, and anti-inflammatory effects (8).

The present study aimed to investigate the pharmacological effects of selective PDE3 inhibitor Cilostazol on hepatic ischemic-reperfusion injury and liver regeneration in a major hepatic resection model created on animals.

Material And Method

The study had an experimental animal design conducted in a laboratory environment. In future surgical procedures, examining the effect of Cilostazol on liver regeneration for the continuation of hepatic functions following hepatectomy may provide new and improved possibilities in treatment. The present study was conducted in Zonguldak Bülent Ecevit University Medical Faculty Medical and Surgical Research Center with the permission of the Experimental Research Ethics Committee with the number B.30.2.Z.K.Ü.0.20.00.00/33.

A total of 40 Wistar Albino rats weighing 200–270 grams, 20 weeks old, were used as experimental animals, and 60% of the liver was resected in all Groups. A total of 4 Groups were formed with ten rats in each. Groups 1 and 2 were chosen as the control groups, and Groups 3 and 4 were the experimental groups. The characteristics of the Groups are given in Table 1.

Table 1
Characteristics of the Groups

Parameters	Group 1, n = 10	Group 2, n = 10	Group 3, n = 10	Group 4, n = 10
Hepatectomy, 60% resection of the liver	+	+	+	+
Cilostazol, 5 mg/kg/day	-	-	+	+
Sacrification, day	4th day	7th day	4th day	7th day

One rat died in each group during the experiment. Anesthesia was provided with 90mg/kg ketamine in rats. Following cleaning, the abdomen was entered with a median incision. The standard hepatectomy technique of Higgins and Anderson was employed for this purpose in which 60% of the liver is resected (9). The Left and median lobes of the liver were resected. The right liver lobe and caudate lobe were left in all rats. Cilostazol was administered daily at 5 mg/kg to the experimental groups. Previous studies reported that mitosis begins 24–30 hours following 60% hepatectomy in rats. Mitosis rate occurs on 4-

7th days at most. In this context, in the present study, the sacrification process was performed on the 4th and 7th days to evaluate liver regeneration.

Sacrification was performed with the intracardiac blood collection method. The liver tissue was excised entirely and preserved in 10% formaldehyde for histopathological examinations and tissue enzyme determinations. Aspartate Aminotransferase (AST, U/L) and Alanine Aminotransferase (ALT, U/L) were studied from sera obtained from the rats. BioSystems Reagents and Instruments kits (BioSystems S.A., Barcelona, Spain) were used. Myeloperoxidase (MPO, U/gr) and superoxide dismutase (SOD, U/mg) activity were measured from tissue enzyme levels (10–11). Advanced Oxidation Protein Products (AOPPs, µmol/L), protein determination (mg/mL), and total sulfhydryl content were calculated over the GSH curve (µmol/mg) (12-13-14).

From morphological parameters, Relative Liver Weight Measurement (RLW) was calculated as liver weight at autopsy - (whole liver weight - resected liver weight) x 100, Mitosis Index (MI) from histopathological parameters, and the rate of labeling with Proliferating Cell Nuclear Antigen (PCNA) (15).

Statistics

The SPSS 22.0 statistical software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics mean ± Standard Deviation (S.D.) descriptive analysis measures were used for patient characteristics and variables. The Mann-Whitney U test compared the groups because parametric test assumptions were not provided, and the $p < 0.05$ value was considered significant.

Results

The present study was conducted on Wistar Albino rats (n = 40). Groups 1 and 2 were the control group in the study. The rats in these groups were not given Cilostazol. Groups 3 and 4 were the experimental groups. The rats in these groups were administered daily Cilostazol at a 5 mg/kg dose. The experimental and control groups (Groups sacrificed on day 4 and Groups sacrificed on day 7) were compared, and the experimental group (Group 3, Group 4) and the control groups (Group 1, Group 2) were also compared.

The liver enzymes ALT and AST levels were statistically insignificant when the experimental and control groups were compared. However, the AST value was lower in the experimental groups. When the experimental and control groups were compared, ALT values were insignificant. In terms of AST, when the control groups were compared, the AST value was lower in Group 2 and was statistically significant. The findings are given in Tables 2 and 3.

Table 2
ALT, AST, tissue enzymes (MPO, SOD), oxidation protein products, total sulfhydryl content (AOPPs, GSH), and histopathological data of the Groups that did not receive Cilostazol (Groups 1 and 2) and Groups that received Cilostazol (Groups 2 and 4).

Parameters	Group 1.n = 10	Group 2. n = 10	Group 3. n = 10	Group 4th n = 10
ALT, U/L	46.5 ± 6.8	43.8 ± 5.6	63.4 ± 6.6	59.7 ± 7.8
AST, U/L	174.4 ± 12.4	158.6 ± 10.4	167.5 ± 13.2	151.7 ± 11.1
MPO, U/ gr	0.20	0.22	0.21	0.13
SOD, U/mg	113.1 ± 24.5	129.1 ± 16.9	146.1 ± 18.5	107.9 ± 23.3
AOPPs, µmol/L	364.7 ± 35.7	603.7 ± 42.7	248.2 ± 36.2	164.03 ± 25.7
GSH, µmol/mg	298.7 ± 26.3	328.1 ± 31.5	343.5 ± 28.3	237.9 ± 28.2
RKA	36.3 ± 13.4	44.1 ± 12.5	41.4 ± 13.3	51.9 ± 12.9
MI, %	3.5	12.6	5.4	19.3
Even nucleus, %	15.4	18.6	17.8	27.3
PCNA, %	15.2	26.6	21.9	39.3

Table 3
Statistical comparison of ALT, AST, tissue enzymes (MPO, SOD), oxidation protein products, total sulfhydryl content (AOPPs, GSH), histopathological data of the groups that did not receive Cilostazol (Groups 1 and 2), and groups that were administered Cilostazol (Groups 2 and 4) results

Parameters	**Group 1 and Group 3** *(p value)*	**Group 2 and Group 4** *(p value)*	**Group 1 and Group 2** *(p value)*	**Group 3 and Group 4** *(p value)*
ALT, U/L	0.113	0.114	0.161	0.489
AST, U/L	0.796	0.120	0.019	0.863
MPO, U/ gr	0.863	0.001	0.666	0.003
SOD, U/mg	0.001	0.136	0.002	0.024
AOPPs, µmol/L	0.040	0.001	0.666	0.011
GSH, µmol/mg	0.161	0.063	0.605	0.031
RKA	0.297	0.161	0.113	0.436
MI, %	0.043	0.004	0.001	0.001
Even nucleus, %	0.297	0.011	0.094	0.001
PCNA, %	0.006	0.001	0.001	0.001
• $p < 0.05$ was taken as the limit of significance.				

When the experimental and control groups were compared in SOD activity, SOD activity was statistically significantly higher in Group 3. When the control groups were compared, SOD activity was significant in Group 2. When the experimental groups were compared, SOD activity was significant in Group 4. When the experimental and control groups were compared in MPO, MPO was significantly lower in Group 4. When the experimental groups were compared, the MPO value was significantly higher in Group 3. When the experimental and control groups were compared in AOPPs, the AOPPs values in Group 3 and Group 4 were statistically significantly lower than in the control group. When the experimental groups were compared, the AOPPs value in Group 4 was significantly lower. When the experimental and control groups were compared in GSH value, the GSH value was significantly lower in Group 4. When the experimental groups were compared, the GSH value was significantly lower in Group 4. Other findings in the comparisons were insignificant. The results are given in Tables 2 and 3.

No significant differences were detected between all groups in terms of the relative weight of the liver. In terms of histopathological mitosis index, the experimental groups (Groups 3 and 4 treated with Cilostazol) had a statistically significantly higher Mitotic Index than the control groups (Groups not treated with Cilostazol; Groups 1 and 2) (Image 1). When the experimental groups were compared, a more

statistically significant Mitosis Index was found in Group 4 and Group 2 than in the control Groups. When the experimental and control groups were compared, double nuclei were significantly more common in Group 4 (Image 2). Cilostazol increased the Mitosis Index and the number of double nuclei. The PCNA ratio was significantly higher in the experimental groups (Group 3, Group 4) (Image 3). When the experimental groups were compared, a higher PCNA rate was found in Group 4 and Group 2 when compared to the control groups. Other findings in the comparisons were insignificant. The results are given in Tables 2 and 3.

Discussion

It is well established that the liver has an extraordinary capacity to regenerate and regain its former functional abilities after extensive resections [16]. The extent of the regenerative process depends on the size and the amount of resected tissue. Therefore, an experimental 60% partial hepatectomy model reveals a well-regulated liver cell proliferation process modulated by various cytokines and growth factors (17). It was shown that regeneration in the remaining liver tissue starts from the first day following partial hepatectomy. Significant regeneration occurs in the first 10 days. The present study was planned in line with this clinical information.

In the present study, it was found that there were positive effects on ischemic reperfusion and increased rates of regeneration markers such as mitosis index, double nucleus, and proliferating cell nuclear antigen in the Groups administered Cilostazol. In this context, the study showed that the selective phosphodiesterase III inhibitor Cilostazol can increase liver regeneration following 60% liver resection.

No statistically significant differences were detected in relative liver weights and ALT and AST values in rats treated with Cilostazol compared to the control group without Cilostazol. In rats administered Cilostazol, there was a significant increase in relative liver weight, although not statistically significant. In a previous study, AST level was shown to be a good clinical indicator of ischemic-reperfusion injury (18–19). In the present study, AST was lower in rats treated with Cilostazol.

Considering the pharmacological effects of PDE3 inhibitors, they can affect the ischemic-reperfusion injury and liver regeneration process. A previous study showed that the PDE3 inhibitor amrinone improved liver regeneration in a rat model with 70% liver resection. The authors hypothesized that this might be because of improved hepatic blood flow and sinusoidal perfusion (20). They also found increased mitosis index, double nucleus, and proliferating cell nuclear antigen ratio, similar to the present study in which it was found that hepatocellular proliferation caused the most significant increase on the 7th day following hepatectomy. It was also found that Cilostazol significantly increased hepatocellular proliferation in rats treated with Cilostazol compared to the control group without Cilostazol.

Following major hepatectomy, elevated portal venous perfusion may result in a critical reduction in hepatic artery perfusion. This may cause ischemia-reperfusion injury and acute liver failure in the liver (21–22). In the present study, SOD activity was significantly higher, and GSH levels, MPO, and AOPPs levels were significantly lower in the groups treated with Cilostazol. These findings can be explained by

the selective inhibition of PDE3 by Cilostazol, which improves hepatic circulation. A previous study shows that PDE3 inhibitors reduce hepatic ischemic-reperfusion injury in animal models (23). This study showed for the first time that the selective PDE3 inhibitor Cilostazol has positive effects on liver hemodynamics.

The widely accepted opinion in ischemia-reperfusion injury following extended hepatic resection is that increased shear stress induces endothelial cell damage during hepatic hyperperfusion. We think that although liver blood flow increased following hepatectomy in rats treated with Cilostazol, shear stress decreased because of significant vasodilation. This opinion is supported by others who reported reduced portal venous and hepatic arterial resistance despite increased portal venous, hepatic arterial, and hepatic parenchymal blood flow after the inhibition of PDE5 (24).

Conclusion

The present study found that the selective PDE3 inhibitor Cilostazol affected histopathological parameters positively and increased hepatocellular proliferation following extended liver resection significantly. It was administered that the relative weight of the liver increases and the ischemic-reperfusion injury decreases. In this regard, Cilostazol can improve the treatment of organ donors and the quality and function of liver tissue in liver transplantation.

Declarations

Funding: No funding has been received for this study

Competing interest: Authors confirm that there are no relevant financial or non-financial competing interests to report

Contribution: E.A and O.O designed the study. G.C and E.A analyzed data. O.O and H.E approved the data and analysis. H.E. and Z.E. wrote the main manuscript text. All authors reviewed the manuscript.

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Figures

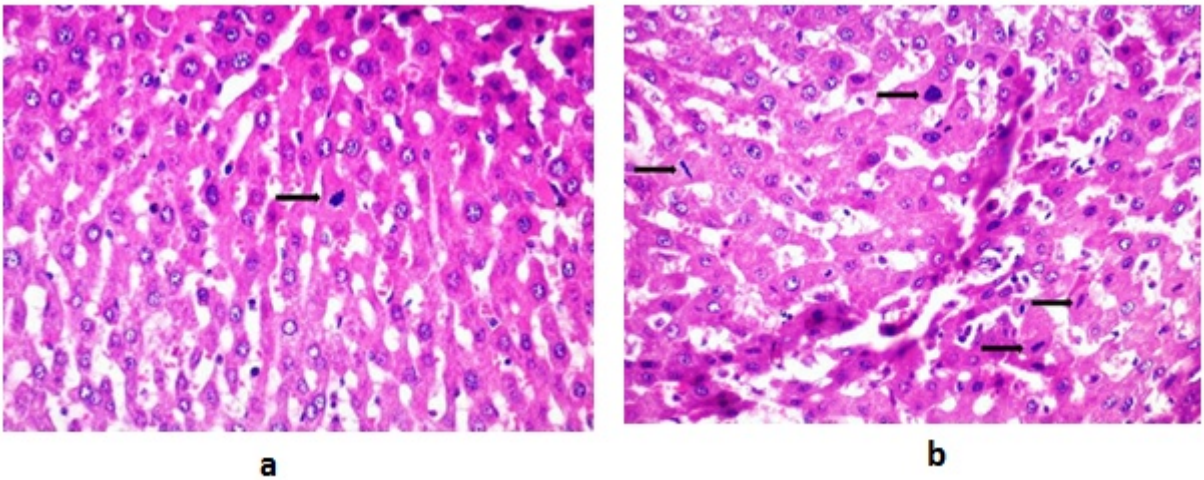

Figure 1

Histopathological staining with Hematoxylin-Eosin (200x), mitosis index (a) in Group 1 from the Groups that did not receive Cilostazol, and mitosis index in Group 3 from the Groups that received Cilostazol (b).

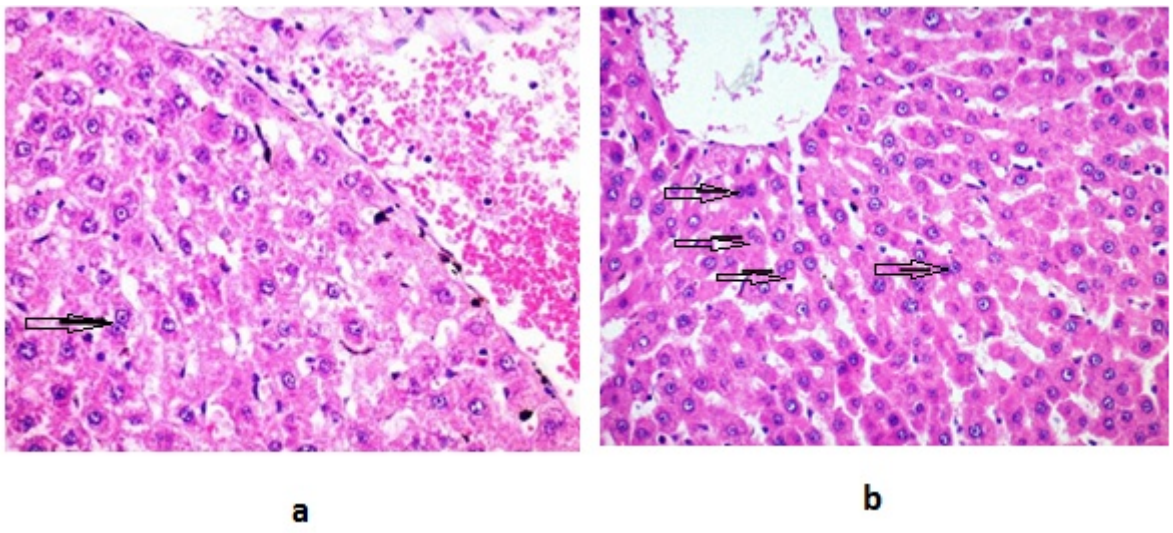

Figure 2

Histopathological staining with Hematoxylin-Eosin (200x), even nucleus (a) in Group 2 from the Groups that did not receive Cilostazol and even nucleus (b) in Group 4 from the Groups that received Cilostazol.

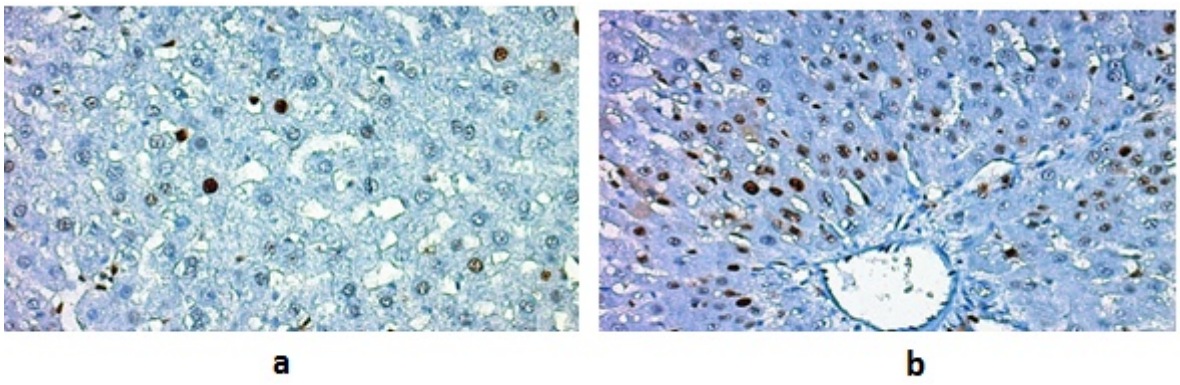

Figure 3

PCNA ratio (a) in Group 1 from the Groups that did not undergo Cilostazol in immunohistochemical staining with the PCNA (Proliferating Cell Nuclear Antigen) (Thermo Scientific, Fremont, CA, USA) kit and the PCNA ratio in Group 3 from the Groups that did not receive Cilostazol (b).