JOURNAL OF CLINICAL MEDICINE OF KAZAKHSTAN (E-ISSN 2313-1519)

Original Article DOI: https://doi.org/10.23950/jcmk/14494

Investigation of the effects of cilostazol on the myocardial ischemia-reperfusion injury of rats

Anar Amrah[1]

[1]*Republican Diagnostical Center, Baku, Azerbaijan*

Received: 2023-10-12.
Accepted: 2024-04-08.

This work is licensed under a Creative Commons Attribution 4.0 International License

J Clin Med Kaz 2024; 21(2): 59-65

Corresponding author:
Anar Amrah
E-mail: anaremrah@gmail.com.
ORCID: 0000-0003-0531-5880.

Abstract

Background. Myocardial ischemia, occurring as a consequence of imbalance between oxygen supply and demand, causes a rapid metabolic and structural impairment within the tissue. After a period of ischemia, sudden onset of reperfusion causes a transition to aerobic metabolism within living cells. Afterwards, emerging substrates initiate a chain of reactions leading to tissue injury. This situation is called "ischemia reperfusion injury". Despite all technical advancements in anesthesia, myocardial protection and cardiac surgical techniques, we still face the clinical reflections of ischemia reperfusion (IR) injury.

Materials and methods. The protective effect of cilostasole on IR injury in an animal model of experimental myocardial ischemia and reperfusion was investigated. In this regional myocardial ischemia model, male Wistar-Albino rats were used as subjects and they were allocated into three groups; ischemia (n=8), sham (n=8), and cilostazole (n=8). LAD was occluded for 45 minutes, and then reperfused for three hours. Rats received Cilostazole 20 mg/kg/day by gastric gavage once daily. During IR hemodynamic parameters were recorded. Serum analysis for CK-MB and Troponin T were analysed at 180th minute of ischemia.

Results. Before the onset of LAD occlusion, as well as at 25th, 60th and 120th minutes of occlusion, all groups were similar in terms of blood pressure and pulse rate.

This study evaluated biochemical markers, energy metabolism, and the antioxidant system in rats with myocardial ischemic damage. Acute ischemia followed by reperfusion led to significant increases in Troponin I and CK-MB levels, indicating myocardial injury. Cilostazol did not significantly affect this process, but it notably inhibited Neopterin synthesis, potentially reducing inflammation. Moreover, cilostazol intake inhibited ADMA synthesis, increasing NO levels, which could alleviate microcirculation disturbances. Cilostazol also weakened lipid peroxidation by enhancing GSH-Px enzyme levels, reducing MDA levels. Overall, cilostazol shows promise in increasing myocardial tolerance to ischemia and protecting against reperfusion damage, suggesting its potential clinical utility.

Conclusion. This study explored how cilostazol affects myocardial ischemia-reperfusion injury in rats, finding that cilostazol administration during

reperfusion may protect against such injury. Through various analyses, we observed positive outcomes associated with cilostazol treatment, suggesting its potential in reducing myocardial damage. Further research is needed to understand the underlying mechanisms and optimize therapeutic strategies, but our findings highlight cilostazol's promise in improving clinical outcomes in cardiac interventions.
Keywords: cilostazol, Myocard, İschemia reperfusion injury.

Introduction

Cardiac surgery is usually performed under bloodless and motionless conditions of the heart. To achieve such conditions, there is a need for global ischemia of the heart. However, while performing global ischemia of the heart, unwanted events can occur. After 20 minutes of a bloodless period, irreversible myocardial damage (ischemic necrosis) occurs [1]. Histopathological examinations of myocardial tissue have revealed that 4 hours after myocardial infarction, coagulative necrosis, edema, and neutrophil infiltration can be detected. Death of myocardial cells occurs during myocardial infarction and also in the reperfusion period when blood flow is restored to the infarction area. Such cell damage can cause arrhythmias, myocardial stunning, and an increase in damaged tissue (infarction). Myocardial damage can be considered an important cause of low cardiac output, which can lead to mortality [2].

The main reasons for ischemia-reperfusion damage include the occurrence of free oxygen radicals, imbalance of Ca2+ ions in myocytes, stimulation of neutrophil accumulation, and formation of adhesion molecules as a result of the release of cytokines and interleukins by endothelial cells or macrophages [3, 4].

Cilostazol, chemically known as 6-[4-(1-cyclohexyl-5-tetrazole) butoxy]-1, 2, 3, 4-tetrahydro-2-oxoquinoline, has the abilities of vasodilation and anti-platelet aggregation. It mainly increases the concentration of cyclic adenosine monophosphate (cAMP) or releases adenosine diphosphate (ADP) and 5-hydroxytryptamine (5-HT) by inhibiting the activity of phosphodiesterase (PDE) in platelets and vascular smooth muscle or the production of thromboxane A2 (TXA2) in the phospholipid membranes [5, 6].

Cilostazol is commonly employed for treating intermittent claudication and occasionally for intracranial atherosclerosis or stroke prevention. Accumulating evidence suggests that cilostazol may serve as a safeguard against ischemia-reperfusion injury in diverse organ systems. Another study indicated the utility of cilostazol pre-treatment in cold hepatic ischemia-reperfusion injury, attributing its efficacy to the prevention of endothelial inflammation and apoptotic death. Additionally, cilostazol pre-treatment enhanced neurological functional outcomes [7, 8].

The purpose of this investigation is to study the effects of cilostazol in ischemia-reperfusion injury and to investigate biochemical, hemodynamic, and histopathological data.

Materials and methods

In the study, 24 male Wistar Albino rats, bred in Gulhane Military Medical Academy Multi-Discipline laboratories, were utilized. General physiological information is provided in Table 1. Rats were selected as experimental animals due to their utility in myocardial ischemia-reperfusion models and their low myocardial coronary collateral circulation [9].

Table 1 Physiologic values of rats.

Physiological charasterictics	Mean value
Average lifespan	2.5-3.5 years
Weight	200-300gr
Body temperature	35.9 - 37.5 ^{0}C
Respiratory rate	100-150/ min
Blood pressure	88-184 mmHg
Blood volume	1/20 of body weight
Heart rhythm	250-450 (240)/min
Hemoglobin	16-19
Hematocrit	0.1gr/100ml
Sodium	320mgr/100mg
Potassium	17.5-22.0 mgr/100ml

The rats were housed at room temperature, with five rats per cage, in a clean environment, under standard laboratory conditions, and were provided with pellet feed. Feeding was halted 12 hours before the surgical procedure, and access to water was removed. After the experiment, the rats were euthanized, and no postoperative care was administered.

Experimental Groups

Group 1 (8 pieces): Sham group. These animals have been fed with water for 14 days with the help of gavage. After the intervention and surgical procedure performed on the subjects, an intramyocardial suture passed on the left anterior descending (LAD) coronary artery but was not occluded with the help of a snare.

Group-2 (8 pieces): Ischemia-reperfusion group. These animals have been fed with water for 14 days with the help of gavage. After the intervention and surgical procedure performed on the subjects, an intramyocardial suture passed on the LAD artery and was occluded with the help of a snare. After 45 minutes of occlusion of the LAD artery, the snare was taken out and reperfusion was performed for 180 minutes.

Group-3 (8 pieces): cilostazol group. These animals have been fed with cilostazol 20 mg/kg/day for 14 days with the help of gavage. After the intervention and surgical procedure performed on the subjects, an intramyocardial suture passed on the LAD coronary artery and was occluded with the help of a snare. After 45 minutes of occlusion of the LAD artery, the snare was taken out and reperfusion was performed for 180 minutes

We neutralized the effects of all interventions and surgical procedures on the hemodynamic, biochemical, and pathological data examined in the study by using a sham group.

Anesthesia and Monitoring

Anesthesia was induced with 35mg/kg ketamine (Ketalar® vial 100mg/mL Alfasan International Holland) and 5mg/kg Xylazine (Alfazyne® 20mg/mL Alfasan International Holland). The dose was repeated when necessary.

The neck and anterior chest wall were shaved and the surgical field was stained with 10% povidone-iodine solution (Isosol Solution Merkez Labaratuvarı A.Ş). Tracheostomy was opened through the neck incision and intubated. They were connected to a mechanical animal respirator (Datex-Ohmeda Excell 410) at 60 respiratory rate/min, 40% oxygen supply, 1,5 mL/150 gr tidal volume.

The carotid artery was for continuous pressure monitoring and the jugular vein was catheterized with a 24G branula.

Surgical Procedure

A left thoracotomy was performed to access the heart. The thorax was entered through the 4th intercostal space. The surgical manipulation area was widened with a mini thorax retractor and the heart was accessed by cutting the pericardium (Figure 1).

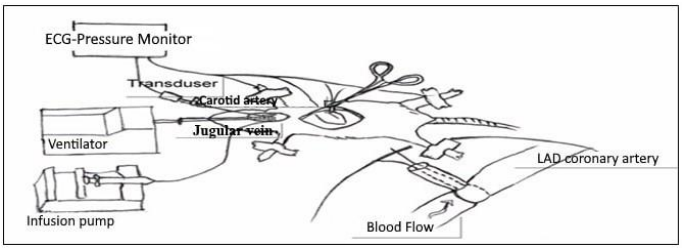

Figure 1 – Schematic drawing of the operation site

A 5-0 10 mm atraumatic needle prolene suture was passed intramyocardially through the LAD branch of the left main coronary artery, which continues in the interventricular septum, proximal to the diagonal side branch.

At the beginning of the 15-min equilibration period, 150IU/kg heparin (Nevparin® vial 5000IU/mL Mustafa Nevzat İlaç San. A.Ş.) was administered intravenously to prevent thrombosis in the coronary artery. At the end of this period, the suture needles were passed through the pledget and the suture threads were tightened with the help of a sner to prevent LAD compromise and to achieve complete occlusion and ischemia. Before suture tightening, 0.5mL of blood was collected for cardiac enzymes (creatine kinase-myocardial band – (CK- MB)). Fluid administration was replaced with Ringer's lactate, 3 times the blood lost during the procedure. During the 45-minute ischemia period, arrhythmias were recorded. At the end of this period, the singer was loosened and reperfusion of the ischemic area was achieved. The experimental animal was kept in reperfusion by a respirator for 3 hours. During this time, the thoracotomy incision was approximated with a temporary prolene suture to minimize insensible loss. At the end of three hours, the rat heart was removed and placed in an empty pathology dish. The pathology container was placed in a cold water-ice mixture and taken to the laboratory of the Department of Pathology without losing time (within 3-4 minutes).

Biochemical Measurements

For the measurement of the cardiac enzyme creatinine kinase-MB (CK-MB), blood was collected before (0 min) and after (45 min) coronary artery occlusion, as well as at the first and third hours following reperfusion. Blood was centrifuged at 5000g at 4°C for 15 minutes. The serum portion was removed and stored at -71°C. After samples were taken from all animal groups for biochemical studies, the Immulite® Turbo CK-MB kit (EURO/DPC Ltd. UK) was used in the Gulhane Military Medical Academy Central Biochemistry Laboratory.

Statistical Method

SPSS for Win. Ver. 15.0 (SPSS Inc. Chicago II., USA) program was used for statistical analysis. Kruskal-Wallis Test was used for statistical comparison between groups, Mann-Whitney U Test was used when a statistical difference was found and Wilkcoxon Signed Ranks Test was used to compare the differences of intragroup values according to baseline values. Statistical results with $p<0.05$ (95% confidence interval) were considered significant.

Results

Since cardiomyocytes lack reserves of energetic substrates, including macroergic energy sources, this study aimed to objectively evaluate the depth of pathological changes in the structural proteins of the myocardium subjected to acute anoxic ischemia. Changes in the levels of creatine phosphokinase (CK-MB) and Troponin I, which are major cardiospecific enzymes, in the blood of rats included in all three groups were studied and comparatively analyzed (Table 2). During this process, it was determined that the levels of CK-MB in the IR and Cilostazol groups, respectively rising to 0.95±0.125 and 0.90±0.010 ng/l, were 4 times higher than the value in the Control group (0.23±0.025 ng/l) (X2=15.905; P<0.001). Notably, despite the equal increase in CK-MB levels in both groups, there was no statistically significant difference between them (P>0.05). A similar trend was recorded in the levels of Troponin I. Although the amount of Troponin I in the blood of animals in the IR and Cilostazol groups increased 10-fold compared to the control, reaching 81.13±2.13 and 82.18±4.69 ng/l (X2=15.560, P<0.001), it was found that there was no statistically significant difference between the comparison groups (P>0.05).

Table 2 Dynamics of CK-MB, Troponin I, Neopterin, and ADMA indicators in the experimental groups.

	Control group (n=8)	İ/R group (n=8)	Silostazol group (n=8)	Statistical indicators
CK-MB (ng/ml)	0.23±0.025) (0.10–0.30)	0.95±0.125 (0.80–1.80)	0.90±0.010 (0.80–1.40)	X^2=15.905 P<0.001
Troponin I (ngr/l)	8.89±0.78 (7.13–13.35)	81.13 ± 2.13 (44.12-61.18)	82.18± 4.69 (53.63–91.21)	X^2=15.560 P<0.001
Neopterin (ng/l)	8.60 ± 0.63 (5.90–11.00)	30.24 ± 2.33 (8.40-26.26)	19.71 ± 1.17 (6.40-15.80)	F=89.019 P<0.001
ADMA (□mol/l)	0.83 ±0.05 (0.57–0.96)	4.80 ± 0.15 (3.76–4.96)	2.38±0.25 (2.46–4.47)	X^2=16.409 P<0.001

Note: *Here and in subsequent tables, X2 denotes Kruskal-Wallis non-parametric variation, F denotes one-way analysis of variance (ANOVA) results, and t denotes Student's t-test criterion. Differences between comparison groups were considered statistically significant at P<0.05 or less.*

Thus, the sharp increase in the levels of CK-MB and Troponin I (respectively 4 and 10 times) in the blood of animals with the IR model created indicates that these indicators are highly informative markers for objectively evaluating the changes occurring in the myocardium subjected to acute ischemia and reperfusion. The absence of a significant difference between the

groups suggests that cilostazol does not have a significant effect on this process.

According to literature data, Neopterin is synthesized by macrophages in the vascular endothelium and plays a significant role in the formation of inflammatory reactions due to its strong immunomodulatory effect. It has the property of increasing the tolerance and viability of cells subjected to acute ischemia and reperfusion. Our studies showed that the amount of Neopterin in the blood of rats in the IR and Cilostazol groups increased to 30.24±2.33 and 19.71±1.17 ng/l, respectively (F=89.019, P<0.001), exceeding the level in the control group by 3.5 and 2.3 times. The fact that the level of Neopterin in the Cilostazol group was 10.53 ng/l lower than in the IR group (P<0.001) could be explained by the inhibition of its synthesis by cilostazol. However, the milder manifestation of histomorphological changes in the myocardium in the Cilostazol group compared to the IR group suggests that this decrease is better explained by the enhanced uptake and rapid elimination of Neopterin by cells subjected to anoxic ischemia. The increased tolerance and viability of cells to hypoxia provided by Neopterin may be achieved in this way.

In addition, the dynamics of asymmetric dimethylarginine (ADMA) in the blood of rats with the IR model were studied. As is known, ADMA is an inhibitor of the enzyme NO synthase, which regulates the synthesis of nitric oxide (NO) from arginine. Considering that NO has strong vasodilator, antiplatelet, antiadhesive, and antioxidant effects, it is clear how important it is to study the effect of cilostazol on the level of ADMA. The results of our studies (Table 2) showed that the amount of ADMA in animals with the IR model increased more than 6 times (up to 4.80±0.15 mmol/l) compared to the control. In contrast, it was observed that this increase was more moderate in rats treated with cilostazol (up to 2.38±0.25 mmol/l) (P<0.001). This means that during the creation of the myocardial IR injury model, a sharp increase in the amount of ADMA in the blood, by inhibiting the synthesis of NO, leads to spasm of microcirculatory vessels in the damaged area, aggregation of platelets, adhesion of monocytes and erythrocytes, thereby creating a basis for severe histomorphological disorders. Moreover, the use of cilostazol before the creation of the IR model significantly prevented the dangerous increase in the amount of ADMA in the blood, thereby eliminating the inhibition of NO synthesis.

The study of the amount of NO in myocardial tissue exposed to IR injury confirmed this conclusion once again (Table 3). It was observed that in the myocardial tissue taken from rats with the IR model, the amount of NO was more than twice that of the control group (15.46±6.25 mmol/l), while in the Cilostazol group, this amount was recorded to be 5.5 times higher, at 84.37±11.12 mmol/l (X2=12.635, P=0.02). In our opinion, this significant difference between the randomized groups based on the main parameters can only be attributed to the effect of cilostazol. As we have shown above, this is based on the effective inhibition of the synthesis of ADMA, an inhibitor of NO synthase, due to the prior use of cilostazol.

If the basis of irreversible myocardial damage during anoxic ischemia is the disruption of membrane permeability and the breakdown of structural proteins, then in reperfusion injuries, endothelial dysfunction, disturbances in the microcirculatory system, and the acceleration of the process of free radical oxidation of lipids come to the forefront. Considering this, the activity of the enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which play a leading role in regulating lipid peroxidation, and the level of malondialdehyde (MDA), the final product of this process, were comparatively studied in myocardial tissue taken from rats in all three groups (Table 3).

As we know, SOD is an enzyme with antioxidant effects, catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide, thus protecting the organism from their toxic effects. Our studies showed a significant increase in the level of SOD in the myocardial tissue of animals in both the IR and Cilostazol groups. Specifically, while the amount of SOD in the Control group animals was 67.28±4.77 ng/l, in the comparison groups, this indicator increased to 118.26±5.75 and 100.64±6.90 ng/l, respectively (F=5.910, P=0.009). The lack of a statistically significant difference in SOD levels between the comparison groups (P>0.05) indicates that the increase in SOD levels in the blood during extreme conditions is part of the body's universal defense system and is not influenced by the effect of cilostazol.

Table 3 Dynamics of key indicators of free radical oxidation of lipids in the comparison groups.

	Control group (n=8)	İ/R group (n=8)	Silostazol group (n=8)	Statistical indicators
NOx (□mol/l)	6,34±2,27 (0,21–20,11)	15,46±6,25 (4,22–54,33)	84,37±11,12 (9,88–98,76)	X^2=12.635 P=0,02
SOD (ng/ml)	67,28±4,77 (53,60–91,80)	118,26±5,75 (93,77–139,8)	100,64±6.90 (69,86–125,18)	F=5.910 P=0,009
GSH-Px (ng/l)	9,94±0,40 (8,65–11,88)	11,41±1,13 (7,59–16,60)	24,27±4,38 (10,28–45,42)	X^2=7.440 P=0,02
MDA (ng/l)	0,14±0,022 (0,09–0,27)	0,88±0,027 (0,76–0,98)	0,34±0,04 (0,17–0,49)	X^2=19.046 P<0.001

In contrast, the changes in the level of MDA, the final product of lipid peroxidation, were more striking (Table 3). Specifically, the amount of MDA in the blood of rats with the IR model increased more than 6 times (from 0.14±0.022 to 0.88±0.027 ng/l) compared to the control group (X2=19.046, P<0.001), which we evaluated as a result of the dangerously accelerated processes of free radical oxidation of lipids under the influence of acute ischemia and reperfusion. In this case, considering that the increase in the amount of MDA observed in the Cilostazol group was significantly weaker (up to 0.34±0.04 ng/l) and 2.6 times lower than the level in the IR group (P<0.001), it can be concluded that the use of cilostazol before creating the IR model prevents the dangerous acceleration of the free radical oxidation process of lipids.

While there was no significant difference in the level of SOD between the comparison groups, when investigating the mechanism behind the amount of MDA in the Cilostazol group being 2.6 times lower than that in the IR group, our attention was

drawn to the changes in the level of the enzyme glutathione peroxidase (GSH-Px), another important element of the antioxidant system. It was found that while the amount of this enzyme in the blood of rats with the IR model did not differ from the level in the control group (9.94±0.40 and 11.41±1.13 ng/l, P>0.05), it increased to 24.27±4.38 ng/l in animals treated with cilostazol (X2=7.440, P=0.02). It can be assumed that the significantly lower amount of MDA in the Cilostazol group compared to the IR group is due to the more than twofold increase in the level of this enzyme in the blood under the influence of cilostazol. While SOD breaks down superoxide radicals into hydrogen peroxide and water molecules, the primary function of the GSH-Px enzyme is to inactivate hydrogen peroxide by breaking it down into water and molecular oxygen, thereby completing the process. Therefore, the prevention of excessive MDA increase in the Cilostazol group is due to this enzyme (Table 3).

Discussion

Transient ischemic attacks for myocardial ischemia-reperfusion injury may occur during severe coronary artery spasm, atherosclerotic plaque rupture, or unmet O2 demand during exertion. The return of myocardial systolic-diastolic function within hours or even days, as in the case of thrombolytic therapy, cessation of exertion, restoration of cardiac circulation after cardiopulmonary bypass, or resolution of coronary artery spasm, is called "myocardial stunning" [10]. Myocardial stunning is associated with prolonged low-output cardiac syndromes and precipitates heart failure.

The molecular events of ischemia-reperfusion injury are complex and multifactorial. In many studies, free oxygen radicals, excessive intracellular calcium accumulation, and the inflammatory response cascade, especially neutrophils, have been shown as possible mechanisms [11-15].

Many agents have been used in experimental studies to prevent myocardial ischemia-reperfusion injury. The main ones are Ca channel blockers, ACE inhibitors, prostaglandins, glutathione, N-acetylcysteine, pentoxifylline, and anesthetic agents.

In the liver ischemia model of Wakabayoshi et al., laser Doppler and histological studies showed that cilostazol given 30 minutes before warm ischemia rapidly restored perihepatic microcirculation. In this study, cilostazol was shown to exert its effect by inhibiting the expression of endothelin 1, a potent vasoconstrictor [16].

Sarc et al. reported that cilostazol given before ischemia prolonged survival and improved the restoration of hepatic ATP content in a model of liver ischemia and hepatectomy in rats [17].

Zini et al. showed that cilostazol inhibited two different complexes (complex 3 and complex 5) in the electron transport chain in studies on mitochondria in a rat brain ischemia model. These are responsible for free oxygen radical formation [18].

The rapid decrease in cellular ATP content during ischemia compromises the electrolyte gradient between intracellular and extracellular compartments. Intracellular Ca+2 is a critical indicator. Dhar et al. showed that intracellular Ca+2 accumulation and hepatocellular damage decreased after reperfusion in a canine liver ischemia model by administering cilostazol [19].

Thus, the comparative analysis of some biochemical markers, energy metabolism, and the state of the antioxidant system in the blood samples of rats subjected to ischemic damage of the myocardium in experimental groups, including those created with IR model and those that received cilostazol two weeks prior, provided important scientific and experimental results. It was found that the myocardial damage due to acute ischemia for 45 minutes and reperfusion for 180 minutes leads to a sharp increase in the levels of Troponin I and CK-MB in the blood compared to the control group. At this time, the lack of significant difference between the IR and Cilostazol groups suggests that cilostazol does not effectively protect against structural damage from ischemia and reperfusion. However, the significant inhibition of Neopterin synthesis by cilostazol can be considered as a positive quality, weakening the inflammatory response in the endothelium and myocardial tissue exposed to acute ischemia and reperfusion. Additionally, the inhibition of ADMA synthesis, an inhibitor of NO synthase, by cilostazol intake facilitates a significant increase in blood NO levels compared to the IR group (by 5.5 times), thus providing a substantial basis for the milder character of microcirculation and rheology disturbances observed under reperfusion.

Given that structural lipid oxidation is one of the main contributing factors to ischemia-reperfusion injury, understanding how cilostazol affects this process has yielded promising results. It was found that pre-administration of cilostazol in animals with the IR model does not prevent the increase in SOD levels in the blood but significantly weakens the peroxidation process by increasing the level of GSH-Px enzyme, which is capable of breaking down and neutralizing peroxide radicals by more than 2 times. The lower level of MDA, the end product of this process, in the blood of animals receiving cilostazol compared to the IR group by 2.6 times clearly confirms this result.

Comparative analysis of the results of experimental research leads to the conclusion that cilostazol may be a promising and effective drug for increasing myocardial tolerance to anoxic ischemia and protecting against reperfusion damage during "open-heart" surgeries, thus warranting its widespread clinical application.

Since the formation of ischemia-reperfusion injury is a complex mechanism that triggers each other, more detailed studies with cilostazol and other active substances are needed.

Conclusion

In this study, we investigated the effects of cilostazol on myocardial ischemia-reperfusion injury in rats. Our findings suggest that cilostazol administration during reperfusion may confer protective effects against myocardial ischemia-reperfusion injury. Through biochemical analyses, we observed favorable outcomes associated with cilostazol treatment. These results indicate the potential of cilostazol in attenuating myocardial damage induced by ischemia-reperfusion injury.

While our study adds valuable insights into the protective effects of cilostazol, further investigations are warranted to elucidate the underlying molecular mechanisms and optimize therapeutic strategies. Overall, our findings underscore the importance of exploring novel pharmacological agents, such as cilostazol, to mitigate the detrimental effects of myocardial ischemia-reperfusion injury and improve clinical outcomes in cardiac surgery and related interventions.

Limitations

The study utilized a relatively small sample size of 24

male Wistar Albino rats. While this sample size was suitable for conducting the experiment, larger sample sizes could provide more robust and generalizable results.

Cilostazol was administered at a fixed dosage to the experimental group. Variations in dosage levels or administration regimens could yield different results, and exploring a range of doses may provide a more comprehensive understanding of cilostazol's effects.

While the study provides valuable insights into cilostazol's effects in a controlled experimental setting, correlating these findings with clinical data from human trials is essential to validate its therapeutic potential in patients undergoing cardiac surgery or experiencing myocardial ischemia-reperfusion injury.

Author Contributions: Conceptualization, formal analysis, investigation, methodology, project administration, supervision; validation, visualization, roles/writing – original draft, writing – review and editing, A. A. The author has read and agreed to the published version of the manuscript.

Disclosures: The author has no conflicts of interest.

Acknowledgments: None.

Funding: None.

References

1. Steenberger C, Hill ML, Jennings RB. Volume regulation and plasma membrane injury in aerobic, anearobic, and ischemic myocardium in vitro. Circ Res 1987;57:864-75
2. Writing Committee Members. Gerhard-Herman MD, Gornik HL, Barrett C, Barshes NR, Corriere MA, Drachman DE, Fleisher LA, Fowkes FGR, Hamburg NM, Kinlay S, Lookstein R, Misra S, Mureebe L, Olin JW, Patel RAG, Regensteiner JG, Schanzer A, Shishehbor MH, Stewart KJ, Treat-Jacobson D, Walsh ME, ACC/AHA Task Force Members. Halperin JL, Levine GN, Al-Khatib SM, Birtcher KK, Bozkurt B, Brindis RG, Cigarroa JE, Curtis LH, Fleisher LA, Gentile F, Gidding S, Hlatky MA, Ikonomidis J, Joglar J, Pressler SJ, Wijeysundera DN. 2016 AHA/ACC Guideline on the Management of Patients with Lower Extremity Peripheral Artery Disease: Executive Summary. Vasc Med. 2017 Jun;22(3):NP1-NP43. [PubMed]
3. Niu PP, Guo ZN, Jin H, Xing YQ, Yang Y. Antiplatelet regimens in the long-term secondary prevention of transient ischaemic attack and
ischaemic stroke: an updated network meta-analysis. BMJ Open. 2016 Mar 17;6(3):e009013. [PMC free article] [PubMed]
4. Powers WJ, Rabinstein AA, Ackerson T, et al.. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2019;50:e344–418. 10.1161/STR.0000000000000211 [PubMed] [CrossRef] [Google Scholar]
5. Li, J., X. Xiang, X. Gong, Y. Shi, J. Yang, and Z. Xu. Cilostazol protects mice against myocardium ischemic/ reperfusion injury by activating a PPARgamma/JAK2/ STAT3 pathway. Biomed. Pharmacother. 94:995–1001, 2017. https://doi.org/10.1016/j.biopha.2017.07.143; (PMID: 28810537).
6. Shimizu, T., T. Osumi, K. Niimi, and K. Nakagawa. Physico-chemical properties and stability of cilostazol. Arzneimittelforschung 35(7a):1117–1123, 1985.Shaw LM, Kaplan B, Kaufman D. Toxic effects of immunosuppressivedrugs: Mechanism and strategies for
controlling them. Clin Chem 1996;42:1316-1321
7. Plosker GL and Foster RH. cilostazol: A further update of its pharmacology and therapeutic use in the management of organ transplantation. Drugs 2000;59:323-389
8. Teagtmeyer H, King LM, Jones BE. Energy substrate metabolism, myocardial ischemia, and targets of pharmacotherapy. Am J Cardiol 1998;82:54-60
9. Hickman DL, Johnson J, Vemulapalli TH, Crisler JR, Shepherd R. Commonly Used Animal Models. Principles of Animal Research for Graduate and Undergraduate Students. 2017:117–75. doi: 10.1016/B978-0-12-802151-4.00007-4. Epub 2016 Nov 25. PMCID: PMC7150119.
10. Kloner RA, Jenning RB. Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: part 1. Circulation 2001;104(24):2981-89
11. Murry CE, Richard VJ, Reimer KA, Jennings RB. İschemic preconditioning slows energy metabolism and delays ultrastructural damage
during a sustained ischemic episode. Circ Res. 1990;66(4):913-931
12. Downey JM, Cohen MV, Mitochondrial K channel opening during index ischemia and following myocardial reperfusion in ischemic rat hearts. J.Mol.Cell Cardiol. 2001;33:651-3
13. Chiara M, Daniela G, Maria M, Shiela L. Further evidence that melanokortins prevent myocardial reperfusion injury by activating melanocortin MC3 receptors. Eur. Jour.Pharm. 2003;447:227-234
14. Thomsan JA, Hess ML. The oxygen free radical system: A fundemantal mechanism in the production of myocardial necrosis. Prog Cardiovasc Dis 1986;28:449-462

15. Southorn PA, Powis G. Free radicals in medicine. 1. Chemical nature and biological reactions. Mayo Clin Proc 1998;63:381-89
16. Wakabayashi H, Karasawa Y, Tanaka S, Kokudo Y. The effect of FK506 on warm ischemia and reperfusion injury in the rat liver. Surg
Today. 1994;24:994-1002
17. Sark MF, Zetti GM, Farghali H, Nalessic FH. FK506 amelorites the hepatic injury associated with ischemia and reperfusion in rats.
18. Zini R, Simon N, Morin C, Tillement JR. cilostazol decreases in vitro oxidative phosphorilation of mitochondria from rat forebrain. Life
Sci. 1998;63:257368
19. Dhar DK, Takemoto Y, Uchidae M, Ono T. FK506 maintains cellular calcium homeostasis in ischemia-reperfusion injury of the canine
liver. J Surg. Res 1996;60:142-146