Investigation of the effects of cilostazol(FK506) on the myocardial ischemia-reperfusion injury of rats

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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/d 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. Ischemic zone was measured by dying with Evans Blue and infarct area was measured by dying with triphenyltetrazolium chloride.

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.

The total area, affected area and necrotic area were calculated by using formulas; affected area ratio= affected area/total area X 100, necrotic area ratio = necrotic area/total affected area X 100, necrotic area and affected area ratio = necrotic area /affected area X 100. Affected area and total area ratio was significantly higher in IR group, compared with cilostazole group (t=8.965; p<0.001). Similarly, necrotic area and total area ratio was higher in IR group, compared with cilostazole group (t=8.965; p<0.001). The necrotic area and affected area ratios were similar in IR and cilostazole groups (t=0.245; p=0.810). CK-MB level differences were not statistically significant between two groups (Z=0.382; p=0.721).

Troponin levels were similar between IR and cilostazole groups and the difference was not statistically significant (Z=0.630; p=0.574). Pathological specimens of the heart were scanned for myocytolysis, PMNL and hemorrhage. The difference between mean value of MDA enzyme levels were statistically significant (p<0.001) between all groups. MDA enzyme levels, from higher to lower was IR, cilostazole and Sham group. SOD levels (F=5.910; p=0.009) were significantly lower in Sham group when compared with IR group (p=0.008). The differences between Sham and cilostazole groups and IR and cilostazole gropus were not statistically significant (p=0.008). According to planimetric values and enzyme levels, cilostazole was found to be effective in reducing the ischemic zone, without effecting the necrotic zone in cardiac ischemia reperfusion damage. Therefore cilostazole has protective effects agains ischemia reperfusion damage.

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.

Materıals 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 [12, 14].

| 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).
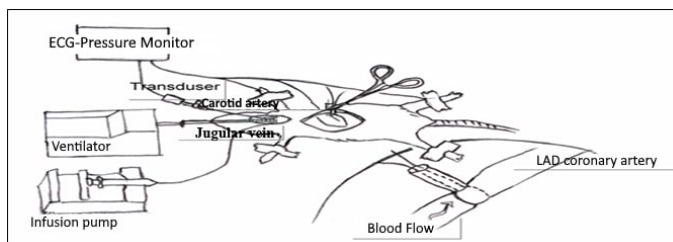
Figure 1 – Schematic drawing of the operation site

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).

Calculation of Infarct Area

In the pathology laboratory, the heart was kept in the refrigerator at -200C for 30 minutes to become semifrozen. During this time, a 1% TTC phosphate buffer solution with a pH of 7.4 was prepared. In this way, the enzymatic activity of the solution was preserved.

The triphenyl tetrazolium chloride (TTC) staining technique reported by Klein et al. was used to calculate the infarct area [23]. Gross examination of the myocardial infarct area showed no change before 12 hours, whereas the necrotic area became visible in tissue sections after 2-3 hours with this method. Since viable cells have dehydrogenase activity, they react with TTC, and the tissue is stained dark red. In ischemic necrotic areas, dehydrogenase enzyme leaks out of the cell due to cell membrane damage and enzyme activity decreases. Since an enzymatic reaction with TTC cannot occur in the infarct area, the tissue remains colorless.

The heart was sliced 3 mm thick from the apex to the base, parallel to the atrioventricular groove from the right ventricle. These slices were kept in saline for 15 minutes to remove blood from the tissue. Then, the sections kept in SF were taken and incubated in TTC phosphate buffer solution at 370C for 30 min. During the incubation, the color difference gradually appeared in the tissues. After the procedure, the sections were kept in 10% formalin solution for 20 min to fix the color difference and photographed. Area calculations were performed in the Gulhane Military Medical Academy Pathology Laboratory using a computerized planimetry program.

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.

Histopathologic Examination

For infarct area calculations, sections were sliced into 4 pieces from apex to base and fixed in 10% formol solution, stained with hematoxylin-eosin stain, and examined for myocardial edema, myocytolysis, focal hemorrhage, and polymorphonuclear leukocyte (PMNL) infiltration under light microscopy.

Pathologic scoring
0-none, 1-mild, 2-moderate, 3-severe.

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

The study was carried out on 8 rats in Sham, IR, and Cilostazol groups, a total of 24 rats. The CK-MB values of the rats in the Sham group ranged between 0.10 and 0.30, while the median was determined as 0.10 (ÇAG=0.10) ngr/l.

CK-MB and Troponin I values according to the experimental groups are given in Table 2 (see the next page).

At least one experimental group differs from the others in a statistically significant amount in the CK-MB enzyme ($x2=15.905$; $p < 0.001$). When investigating which group the difference originates from; It was observed that the Sham group

Table 2 CK-MB, Troponin I, values according to experimental groups

	Sham		IR		Cilostazol		Comparison between groups*	
Biochemical Indicators	Smallest - Largest	Median (ÇAG) AVG±Sd	Smallest - Largest	Median (ÇAG) AVG±Sd	Smallest - Largest	Median (ÇAG) AVG±Sd	Test Statistics	p
CK-MB (ngr/ml)	0.10 – 0.30	0.10 (0.10)	0.80 – 1.80	0.95 (0.60)	0.80 – 1.40	0.90 (0.55)	x^2=15.905	<0.001
Troponin I (ngr/l)	7.13 – 13.35	8.89 (5.15)	61.18 – 94.12	81.13 (21.46)	53.63 – 91.21	82.18 (30.55)	x^2=15.560	<0.001

*x^2: Kruskal-Wallis non-parametric analysis of variance result
F: One-way analysis of variance (ANOVA) result

had a statistically significantly lower CK-MB median than the IR and Cilostazol groups (p<0.001), while the difference between IR and Cilostazol was not significant (Z=0.382; p=0.721). When the CK-MB median is ordered from largest to smallest; IR, Cilostazol, and Sham sequences are formed.

Troponin-t, a myocardial protein, was statistically significantly different from the others in at least one experimental group ($x2$=15.560; p<0.001). When investigating which group the difference originates from; It was observed that the Sham group had a statistically significantly lower Troponin median than the IR and Cilostazol groups (p<0.001), the results between IR and Cilostazol were very close to each other and the difference was not significant (Z=0.630; p=0.574). When the troponin median is ordered from largest to smallest it consists of the order of Cilostazol, IR, and Sham.

The Superoxide Dismutase (SOD) values of the rats in the sham group ranged between 13.60 and 94.80, with an average of 67.28±31.91 U/gpro. SOD, malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) according to the experimental groups are given in Table 3.

Table 3 SOD, MDA, GSH-Px and NOx values according to the experimental groups

	Sham		IR		Cilostazol		Comparison between groups*	
Biochemical Indicators	Smallest - Largest	Median (ÇAG) AVG±Sd	Smallest - Largest	Median (ÇAG) AVG±Sd	Smallest - Largest	Median (ÇAG) AVG±Sd	Test Statistics	p
SOD (U/gpro)	13,60 – 94,80	67,28 ± 31,91	93,77 – 169,88	118,26 ± 31,27	69,86 – 149,18	100,64 ± 26,95	F=5.910	0.009
MDA (mmol/gpro)	0,09 – 0,27	0,14 (0,12)	0,76 – 0,98	0,88 (0,19)	0,17 – 0,49	0,34 (0,19)	x^2=19.046	<0.001
GSH-Px (U/gpro)	8,65 – 11,88	9,94 (2,37)	10,28 – 45,42	24,27 (29,52)	7,59 – 16,60	11,41 (8,33)	x^2=7.440	0.024

*x^2: Kruskal-Wallis non-parametric analysis of variance result
F: One-way analysis of variance (ANOVA) result

At least one experimental group differed from the others in a statistically significant amount in SOD enzyme (F=5.910; p=0.009). When investigating which group the difference originates from; It was seen that the Sham group had a statistically significantly lower mean of SOD than the IR group (p=0.008), the difference between Sham and Cilostazol, and between IR and Cilostazol was not significant (p>0.05). When the SOD median is ordered from largest to smallest it consists of the order of IR, Cilostazol, and Sham.

The median of the MDA enzyme was statistically significantly different from the others in at least one group ($x2$=19.046; p<0.001). When investigating which group the difference originates from; it was seen that all groups were statistically different from each other (p<0.001). When the median of the MDA enzyme is ordered from largest to smallest; It consists of the order of IR, Cilostazol, and Sham.

The median of GSH-Px was different from the others in at least one experimental group ($x2$=7.440; p=0.024). When investigating which group the difference originates from; It was seen that the difference between Sham and IR was significant (Z=2.731; p=0.005), the differences between Sham and

Cilostazol, and between IR and Cilostazol were not significant (p>0.05). When the median of GSH-Px is ordered from largest to smallest it consists of the order of IR, Cilostazol, and Sham.

Total area, affected area, and Necrosis area were calculated from the sections obtained from the hearts of the rats in the experimental groups. Since at least 4 sections were taken from each rat, firstly the mean total area, mean affected area, and mean necrosis area values were calculated for each rat. The total area, affected area, and necrosis area values according to the experimental groups are shown in Table 4.

Table 4 Total, affected and necrosis area (mm2) by experimental groups

Area(mm²)	Exp group	Mean	Std. Deviation	Test Statistics*	p
Total	Sham	74.81	17.91	F=0.419	0.663
	İR	67.31	12.73		
	Cilostazol	72.99	19.83		
Affected	Sham	n/a	n/a	t=4.002	0.001
	İR	10.60	2.30		
	Cilostazol	5.76	2.53		
Necrosis	Sham	n/a	n/a	t=3.149	0.007
	İR	6.94	1.32		
	Cilostazol	4.04	2.25		

*: F: One-way analysis of variance (ANOVA) result
t: students' t test result

The total area did not differ significantly between the experimental groups (F=0.419; p=0.663). This result shows that the rats were randomly distributed to the experimental groups with a balanced mean heart size.

Since no measurement could be made in the Sham group in the size of the affected area (there is no affected area), only the IR and Cilostazol groups were compared. The affected area in the IR group was larger than in the Cilostazol group (t=4.002; p=0.001).

Since no measurement could be taken from the Sham group in the size of the necrosis area (since necrosis did not occur), only the IR and Cilostazol groups were compared. The area of necrosis in the IR group was larger than in the Cilostazol group (t=3.149; p=0.007).

Using the total area, affected area, and necrosis areas obtained from the sections in the study; Affected area ratio = Affected area / Total area x100, Necrosis area ratio = Necrosis area / Total area x100, and Necrosis area ratio to Affected area = Necrosis area / Affected area x100 formulas affected and necrosis area ratios were calculated (Table 5).
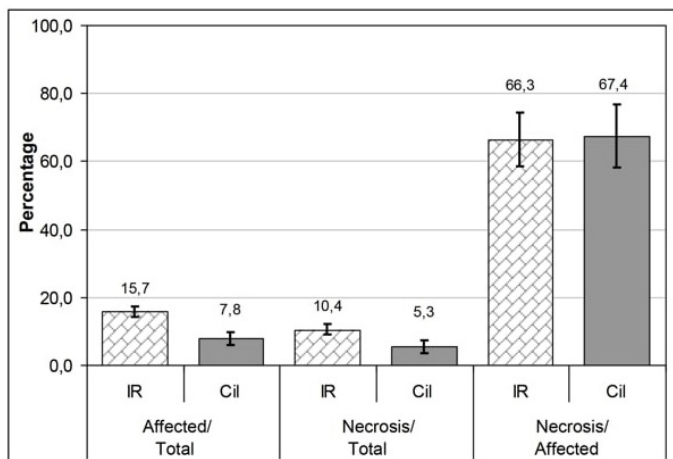
Table 5 Affected area and Necrosis area rates by experimental groups

Ratio(%)	Exp Gr	Mean	Std. Deviation	Test Statistics*	p
Affected Area/ Total Area	İR	15.73	1.60	8.965	<0.001
	Cilostazol	7.80	1.92		
Necrosis Area /Total Area	IR	10.41	1.49	5.870	<0.001
	Cilostazol	5.35	1.93		
Necrosis Area /Affected Area	IR	66.33	7.82	0.245	0.810
	Cilostazol	67.38	9.14		

t: students' t test result

In the ratio of the affected area to the total area, the IR group had a statistically significantly higher mean than the Cilostazol group (t=8.965; p<0.001). Similarly, the IR group had a higher mean than the Cilostazol group in the ratio of necrosis area to total area (t=5.870; p<0.001). There was no significant

difference between the IR and Cilostazol groups in the ratio of necrosis area to the affected area (t=0.245; p=0.810) (Figure 6).

Figure 6 – Affected area and necrosis area ratios by experimental groups (with standard deviation bars)

Histopathologıcal fındıngs

Pathological samples taken from the sections in the study; Edema, myocytolysis, PMNL and hemorrhage were evaluated by the pathologist. One sample from four different sections taken from each rat was evaluated histopathologically. A total of 96 samples were analyzed in the Sham, IR and Cilostazol groups (32 in each group). The histopathology results according to the groups are shown in Table 5-7.

Table 6	Myocardial edema scores according to experimental groups

	None		Mild		Moderate	
Exp grouıp	n	%	n	%	n	%
Sham	26	46.4	6	20.0	0	0.0
IR	15	26.8	11	36.7	6	60.0
Cilostazol	15	26.8	13	43.3	4	40.0
Total	**56**	**100.0**	**30**	**100.0**	**10**	**100.0**

Table 7	Myocytolysis scores according to experimental groups

	None		Mild		Moderate	
Exp group	n	%	n	%	n	%
Sham	27	54.0	5	16.1	0	0.0
IR	12	24.0	11	35.5	9	60.0
Cilostazol	11	22.0	15	48.4	6	40.0
Total	**50**	**100.0**	**31**	**100.0**	**15**	**100.0**

There was a statistically significant difference between the experimental groups in terms of myocardial edema scores (x2=15.410; p=0.004). When analyzing the source of the variance, it was found that there were similar edema scores between the IR and Cilostazol groups (x2=0.567; p=0.753). However, there were notable disparities in edema scores between the Sham and IR (x2=12.798; p=0.002) as well as between the Sham and Cilostazol (x2=11.174; p=0.004) groups. The difference is due to the Sham group. Myocardial edema scores in the sham group were much lower than in the study groups (Table 5).

There was a statistically significant difference between the experimental groups in terms of myocytolysis scores (x2=22.943; p<0.001). When investigating the source of the distinction between groups, it was observed that myocytolysis scores exhibited similarity between the IR and Cilostazol groups (x2=1.259; p=0.533). However, significant differences were noted between the Sham and IR (x2=17.019; p<0.001) as well as between the Sham and Cilostazol (x2=17.737; p<0.001) groups. The source of the difference is the Sham group. Myocytolysis scores in the sham group were much lower than in the study groups (Table 6).

There was a statistically significant difference between the experimental groups in terms of PMNL infiltration (x2=18.262; p=0.001). When investigating the origin of the discrepancy among groups, it was found that PMNL infiltration showed no significant difference between the IR and Cilostazol groups (x2=2.786; p=0.248). However, there were notable variances between the Sham and IR (x2=14.013; p=0.001) as well as between the Sham and Cilostazol (x2=12.553; p=0.002) groups. The difference is due to the Sham group. PMNL infiltration in the sham group was much lower than in the study groups (Table 6).

There was a statistically significant difference between the experimental groups in terms of hemorrhage score (x2=12.384; p=0.015). When assessing the source of the distinction among groups, it was observed that the hemorrhage score exhibited similarity between the IR and Cilostazol groups (x2=2.134; p=0.344). However, there were significant differences between the Sham and IR (x2=10.570; p=0.005) as well as between the Sham and Cilostazol (x2=7.270; p=0.026) groups. The source of the difference is the Sham group. The hemorrhage score in the sham group was much lower than in the study groups (Table 7).

Dıscussıon

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, 12, 13]. 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 [5, 15, 17, 18].

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 [18].

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 [19].

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 [20].

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 [21].

In our study, cilostazol maintained hemodynamic values of blood pressure, decreased PMNL infiltration in the microscopic examination, and decreased infarct area/whole area ratio in the macroscopic image showed that cilostazol may have a protective effect in ischemia-reperfusion injury. We think that our study will shed light on this issue by adding microscopic and macroscopic data to the data on this subject.

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.

Conclusıon

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, hemodynamic, and histopathological analyses, we observed favorable outcomes associated with cilostazol treatment. Specifically, cilostazol maintained stable hemodynamic parameters, reduced neutrophil infiltration, and decreased the ratio of infarcted area to total area. 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.

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