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Original Article

Effects of cilostazol on the intensity of lipid peroxide oxidation (LPO) process in rats with a model of myocardial ischemia-reperfusion (IR) injury

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Abstract

Aim: One of the primary factors in the pathogenesis of morphofunctional processes occurring in the myocardium under anoxic ischemia and reperfusion injury is the formation of highly toxic reactive oxygen species (ROS) and subsequent peroxide oxidation. This oxidative damage results in the breakdown of structural lipids, leading to disrupted transmembrane ion permeability, reduced contractility, dangerous arrhythmias, and hemodynamic disorders. To investigate the changes in levels of the Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px) enzymes and malondialdehyde (MDA) in a myocardial ischemia-reperfusion (IR) injury model in rats, as well as the effects of prophylactic cilostazol on these parameters.
Material and Methods: The study was conducted on 24 Wistar Albino rats divided into three groups of eight animals each. In the control group, no coronary occlusion was performed, while in the IR group, acute ischemia was induced via Left Anterior Descending artery (LAD) compression for 45 minutes, followed by 180 minutes of reperfusion. In the cilostazol group, 20 mg/kg/day of cilostazol was administered via gavage for two weeks before IR induction. Levels of SOD and GSH-Px enzymes and MDA in the blood were evaluated to assess lipid peroxidation (LPO) activity.
Results: The SOD level was 67.28±4.77 ng/l in the control group, rising to 118.26±5.75 ng/l in the IR group and 100.64±6.90 ng/l in the cilostazol group ($P=0.009$); the difference between groups was not statistically significant ($P>0.05$). While the GSH-Px level remained unchanged in the IR group, it more than doubled in the cilostazol group ($P=0.02$). The amount of MDA increased sixfold in the IR group compared to the control ($P<0.001$) but only doubled in the cilostazol group, making it 2.5 times lower than in the IR group ($P<0.001$).
Conclusion: Administering cilostazol before establishing the IR model prevents critical activation of LPO. This antioxidant effect is attributed to increased activity of both SOD and GSH-Px enzymes.

Keywords: Myocardial ischemia-reperfusion injury, reactive oxygen species, lipid peroxidation, cilostazol, antioxidant enzymes, malondialdehyde

INTRODUCTION

Reliable protection of the myocardium from anoxic ischemia and reperfusion injuries during "open heart" operations remains one of the most pressing challenges in cardiac surgery (1-6). Numerous experimental and clinical studies have established that the pathological changes occurring in the myocardium during acute ischemia primarily affect energy exchange (macroergic deficit, glycolysis, acidosis), mitochondria, and cell membranes (7-10). Key factors in the pathogenesis of injuries following the restoration of coronary blood supply (reperfusion) include endothelial dysfunction, vascular spasm, increased platelet reactivity (aggregation and adhesion), microcirculation disorders, activation of inflammatory reactions, and structural lipid peroxide oxidation (LPO) (11-16).

While free oxygen radicals (ROS) are produced during normal cellular respiration, a dynamic balance exists between antioxidant

protection mechanisms and LPO processes, which helps shield the body from the harmful effects of these toxic compounds to some extent (17). Maintaining this balance is crucial for cellular vitality, especially in extreme situations. When the functional capacity of the antioxidant system (AOS) fails to keep ROS levels within physiological limits, a pathological condition known as "oxidative stress" arises (18). Disruption of this balance towards LPO in cells subjected to ischemia-reperfusion leads to the breakdown of membrane lipids under aerobic conditions and the impairment of transmembrane ion permeability, particularly affecting the sensitive ATP/K+ channel (17,19-23).

Given the high metabolic activity of cardiac muscle, even a brief interruption of coronary blood flow during "open heart" surgery can deprive cardiomyocytes of oxygen and energy substrates, resulting in ischemic damage or even necrosis. The balance between LPO and AOS processes is considered critical for improving early outcomes of surgical interventions (15,16,24-27). In this context, investigating the effect of cilostazol on LPO activity to protect the myocardium from ischemic/reperfusion (IR) damage is a significant scientific and experimental issue.

Purpose: The aim of this experiment was to investigate the effect of cilostazol administration on the activity of LPO processes prior to establishing the myocardial IR injury model.

MATERIAL AND METHODS

The studies were conducted on 24 Wistar Albino rats in a single-blind, prospective scientific design utilizing an in vivo ischemia-reperfusion (IR) injury model. The animals were randomized based on baseline parameters into control and comparison groups. This research adhered to the "Principles of Laboratory Animal Care" by the National Institutes of Health (1996) and received approval from the Gülhane Military Medical Academy Ethics Committee (November 2008 – 08/89 K-R).

The experimental animals were divided into three groups of eight. Thoracotomy and pericardiotomy were performed under endotracheal isoflurane anesthesia. The anterior descending branch of the left coronary artery (LAD) was identified in the segment just after the diagonal artery (D1), and a 5/0 Vicryl suture was passed beneath it before the snare was removed.

- **Group I (Control):** In these eight unligated rats, acute ischemia was induced by snare clamping for 45 minutes, followed by reperfusion for 180 minutes.
- **Group II (IR group):** This group consisted of eight rats that were administered 20 mg/kg/day of Cilostazol (Pletal) dissolved in 30% dimethyl sulfoxide via intragastric gavage every morning for two weeks prior to surgery.
- **Group III (Cilostazol group):** The remaining eight rats were included in this group. This methodology is recognized as the most suitable IR model by many researchers and continues to be widely used today (28-32).

During the operation, adequate analgesia was maintained through isoflurane inhalation. The rats were connected to a mechanical ventilator (Datex-Ohmeda Excell 410), with ventilation parameters set according to standards (respiratory rate: 60 per minute, volume: 1.5 ml/150 grams, oxygen concentration: 40%). Invasive monitoring was used to track arterial pressure, ECG, SO2, and body temperature.

At the end of the experiment, blood samples were collected from all animals and stored at 4°C for 15 minutes. The samples were then centrifuged at 5000 RPM for 15 minutes, and the resulting sera were preserved in 500-microliter Eppendorf test tubes at -71°C. The functional status of the AOS was assessed by measuring the activity of the primary enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), along with the levels of malondialdehyde (MDA), a final product of LPO. These examinations were conducted using methods established in the GATA Central Biochemistry Laboratory.

Statistical analysis of the results was performed using MS Excel 2003 and SPSS for Windows (Version 15.0, SPSS Inc., Chicago, IL, USA). The reliability of detected differences was evaluated using one-way analysis of variance for SOD, a non-parametric method (ANOVA) for GSH-Px and MDA, and the Kruskal-Wallis test. A P-value of less than 0.05 was considered statistically significant between comparison groups.

RESULTS

Our experimental studies in rats aimed to objectively assess the balance between lipid peroxide oxidation and antioxidant systems, established a model of myocardial IR injury, and elucidating how Cilostazol affects these processes allowed us to obtain many promising results.

It was determined that the exposure of myocardium to acute ischemia and reperfusion is accompanied by a dangerous acceleration of lipid free radical oxidation processes and a sharp increase in the amount of MDA, the final product of this process (Table 1). As can be seen from the table, the increase in the amount of MDA in the blood of IR model rats more than 6-fold compared to the control group (from 0.14±0.022 to 0.88±0.027 ng/l) clearly confirms this idea ($P<0.001$). In contrast, the increase in the amount of MDA recorded in the cilostazol group (up to 0.34±0.04 ng/l) was 2.6-fold lower than in the IR group ($P<0.001$). We found it necessary to search for the reason for the changes in the activity level of the main enzymes of the antioxidant system, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), in response to their destructive effects. Acute ischemia and reperfusion.

For this purpose, our examinations revealed that SOD activity in the blood increased significantly in both groups of rats with the IR injury model (Table 1). Thus, while the amount of SOD in the blood of the control group was 67.28±4.77 ng/l, it was recorded as 118.26±5.75 ng/l in the IR group and 100.64±6.90 ng/l in the cilostazol group. It was stated that it increased up to

1. Meanwhile, although the SOD level in the IR and cilostazol groups was 76.2% and 50.7% higher than the control group, respectively (P=0.009), it was revealed that there was no reliable difference between them (P>0.05). The reason for the low MDA level in the cilostazol group should be sought in the changes in the activity of the GSH-Px enzyme.

Table 1. The nature of changes in the main indicators of free radical oxidation of lipids in animals included in the comparison groups

Indicators	Control group (n=8)	I/R group (n=8)	Cilostazol group (n=8)	Statistical indicators
SOD (ng/ml)	67.28±4.77 (53.60–91.8)	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 this regard, our investigations showed that this result is correct. It was determined that the amount of GSH-Px enzyme in the blood of IR model rats was not significantly different from the level in the control group (9.94±0.40 and 11.41±1.13 ng/l, respectively; P>0.05). application in animals increased to 24.27±4.38 ng/l, and these indicators were exceeded more than 2 times (P=0.02). This result shows that cilostazol prevents excessive increase in the amount of MDA by significantly weakening the intensity of the lipid peroxide oxidation process in IR model animals in an amount that increases the activity of this enzyme (Section 1).

DISCUSSION

As is known, during extreme conditions such as acute ischemia and reperfusion effects on the myocardium, the activity of SOD in the blood increases. This occurs as SOD breaks down superoxide radicals into hydrogen peroxide (H_2O_2) and oxygen, thereby preventing an excessive increase in superoxide levels (21,23,33). However, the excess H_2O_2 formed during this process can serve as a precursor for the synthesis of other hydroxyl compounds that have stronger toxic effects. Consequently, the primary function of the GSH-Px enzyme is to neutralize H_2O_2 by converting it into water and oxygen. Thus, both physiological and extreme conditions maintain the balance between LPO and antioxidant systems through the increased activity of these two enzymes, which helps in breaking down superoxide radicals that have reached critical levels (21,22).

As previously mentioned, the activation of LPO in extreme cases is primarily indicated by a significant increase in MDA, the final product of this process. Our study demonstrated a sharp rise in blood MDA levels in rats subjected to the IR model. Moreover, cilostazol significantly reduced the intensity of this increase, aligning with findings from other researchers (18,20). In contrast to the more than six-fold increase in blood MDA levels in the IR group (P<0.001) compared to the control group, the cilostazol group exhibited a much weaker increase (P<0.001). This suggests that while cilostazol does not completely inhibit the activation of LPO processes in the myocardium during acute ischemia and reperfusion, it effectively prevents their acceleration to dangerous levels.

To better understand the positive mechanism of action of cilostazol, we investigated its effect on the activity of SOD and GSH-Px enzymes, which are crucial components of the body's antioxidant defense system. Our examinations indicated that although SOD levels increased similarly in both the IR and cilostazol groups (76.2% and 50.7%, respectively), there was no statistically significant difference between the groups. It is worth noting that the literature remains inconclusive regarding cilostazol's effect on SOD activity. In extreme conditions, such as myocardial exposure to acute IR effects, the increase in SOD levels can be seen as part of the body's universal defense mechanism, suggesting that cilostazol does not have a direct effect on this enzyme.

In contrast, the impact of cilostazol on the activity of the GSH-Px enzyme, which provides antioxidant effects, appears to be more significant before the establishment of the IR model. In the IR group, GSH-Px levels did not show a substantial change at the end of the experiment and did not differ from the control group (P>0.05). However, in the cilostazol group, GSH-Px levels increased by more than twofold, reinforcing the notion that cilostazol enhances the antioxidant response prior to IR. Notably, we did not find any information in the literature regarding cilostazol's effect on GSH-Px enzyme activity in laboratory animals used as a model for myocardial IR injury.

CONCLUSION

1. Administration of cilostazol at a dose of 20 mg/kg/day for 2 weeks prior to establishing the myocardial ischemia-reperfusion model in rats significantly reduced lipid peroxidation, resulting in a 2.6-fold decrease in the levels of MDA, the final product of this process, compared to the control group without cilostazol.
2. The antioxidant effect of cilostazol during acute ischemia and reperfusion is attributed not only to the increase in SOD levels in the blood but also to a more than two-fold enhancement in the activity of the GSH-Px enzyme.
3. Based on these findings, cilostazol can be recommended as an effective antioxidant agent to protect the myocardium from ischemia-reperfusion damage in "open heart" surgeries.

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Ethics Committee Approval: Received approval from the Gulhane Military Medical Academy Ethics Committee (November 2008 – 08/89 K-R).

Patient Informed Consent: Not necessary for this manuscript.

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