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# Effect of Iloprost, a Prostacyclin Analogue, on Myocardial Ischemia-Reperfusion Injury

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## ABSTRACT

Myocardial ischemia-reperfusion injury continues to be observed during open heart surgery. Various experimental models have been developed to overcome this injury and to increase postoperative prognosis.

This study was conducted to assess the effect that iloprost, a prostacyclin analogue, can have on myocardial ischemia-reperfusion injury. We evaluated tissue damage by measuring the levels of malonyldialdehyde (MDA), glutathione, and nitric oxide (NO) in tissue and perfusates.

In this study, 20 guinea pig hearts were prepared by using the modified Langendorff perfusion apparatus to form control (n = 10) and experimental study groups (n = 10). Following a preischemic period of perfusion and an ischemic period of 20 minutes, control hearts were perfused with Krebs–Henseleit solution. In the experimental group, iloprost (0.45 µg/kg per hour) was included in the perfusates for the last 10 minutes of the preischemic phase. Following cardiac stabilization, heart rate (pulse/min), contractility (mm), and aortic pressure (mmHg) values were recorded at the end of preischemia, postischemia, and reperfusion. Perfusate and tissue analyses for glutathione, MDA, and NO levels were made in each group at the end of experiments.

Iloprost was found to have protective effects against myocardial ischemia by means of increased myocardial contractility, decreased tissue/perfusate glutathione levels and inhibited rise of tissue/perfusate MDA observed in the iloprost-treated experimental group. Future investigations on myocardial ischemia-reperfusion injury must evaluate iloprost-related mechanisms.

## INTRODUCTION

The cardiovascular system is responsible for transporting oxygen, nutrients, and waste products to and from cells in order to enable cell survival. Nearly 100 trillion cells in the body require a properly functioning cardiovascular system to remain alive, so it is important to prevent any injury to myocardial tissue. Injury to myocardial tissue can arise from ischemia due to coronary artery occlusion or from reperfusion after coronary revascularization.

Myocardial ischemia is a leading cause of death during cardiac surgery, and various measures are taken to reduce this ischemia and thereby decrease mortality and maintain proper cardiac function. Coronary artery bypass grafting (CABG) is one of the most popular cardiac surgical operations, and cardiopulmonary bypass (CPB) is one of the most frequently applied procedures during CABG [Edmunds Jr 2002]. Reperfusion injury, an undesirable but inevitable side effect of CPB, occurs during the return of blood to ischemic tissue and can cause extensive damage to muscle cells [Forman 1990].

There are several structural, functional, and biochemical alterations that arise during reperfusion. These alterations can trigger postoperative arrhythmias that are one of the leading causes of postoperative mortality [Gardner 1983]. Furthermore, during ischemia/reperfusion, polymorphonuclear leukocytes (PMNLs) may overproduce superoxide anions and other oxygen free radicals in the recruited ischemic tissue that has a role in the formation of ischemia-reperfusion injury–related arrhythmias [Das 1986].

Iloprost, an analogue of prostacyclin, has been shown to reduce oxygen free radical production during ischemia/reperfusion via stimulation of cAMP production in the PMNLs. This may help to prevent ischemia-reperfusion injury and related arrhythmias [Simpson 1987] This study was performed

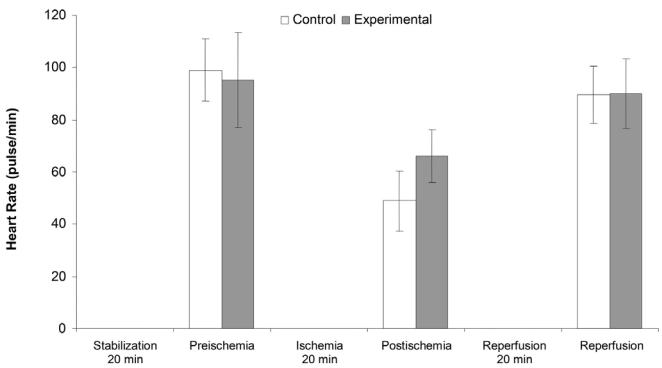


Figure 1. Heart rate (pulse/min) values of control and experimental groups (x ± SD).

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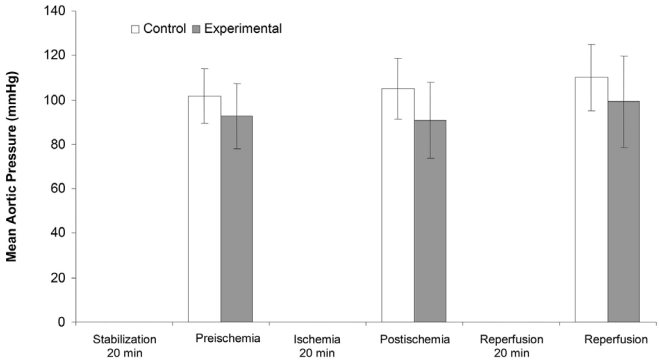


Figure 2. Mean aortic pressure (mmHg) values of control and experimental groups (x ± SD).

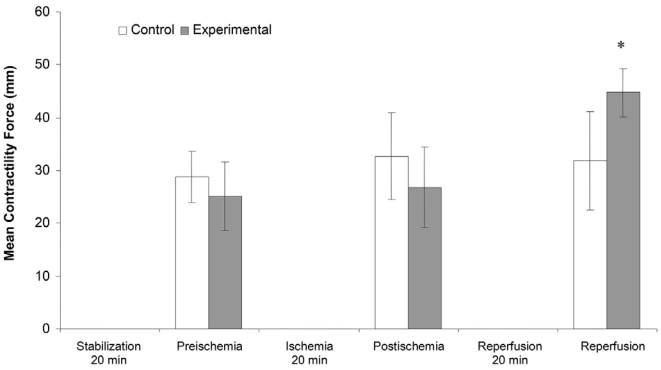


Figure 3. Mean contractility (mm) values of control and experimental groups (x ± SD). *$P$ < .05: Compared to control group.

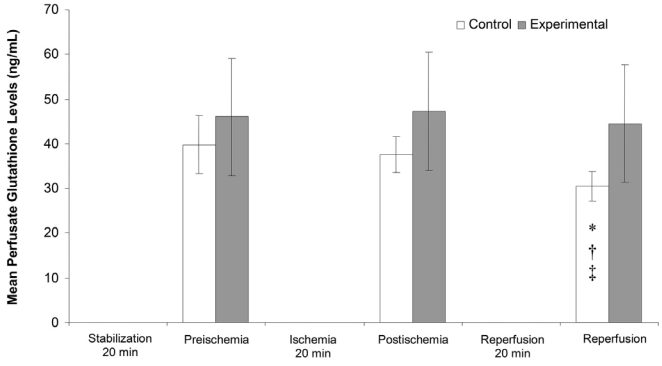


Figure 4. Mean perfusate glutathione values of control and experimental groups (x ± SD). *$P$ < .05: Compared to control groups. †$P$ < .05: Compared to preischemic data. ‡$P$ < .05: Compared to postischemic data.

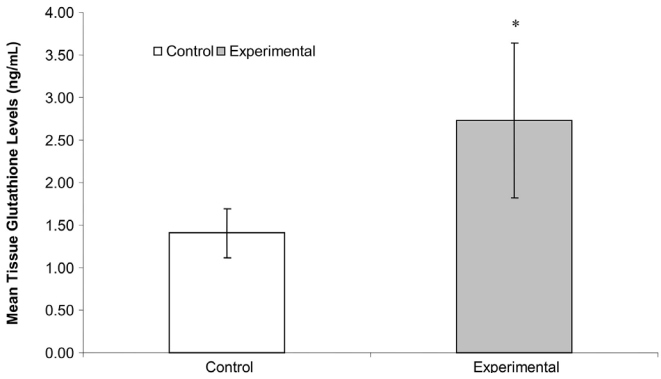


Figure 5. Mean tissue glutathione values of control and experimental groups (x ± SD). *$P$ <.05: Compared to control group.

in order to investigate the effects of iloprost, added to perfusate during CPB, on myocardial ischemia-reperfusion injury in an experimental model of isolated guinea pig hearts.

## MATERIALS AND METHODS

This study was conducted in 20 guinea pigs of both sexes, weighing 300 to 400 g, which were fed a standard chow. Experimental animals were obtained from Gazi University Faculty of Medicine Experimental Animal Research Center (Ankara, Turkey). Guinea pigs were administered heparin (intraperitoneally, 300 unit per kg) and then anesthetized with 25% urethane (intraperitoneally, 0.7 mL/100 g). The surgical procedure was initiated with a midline thoracotomy to reach the mediastinum. Then, the pericardium was detached, and the heart and aorta were isolated and explanted. The ascending aorta was cannulated at a point 0.5 to 1 cm distal to its origin, and the exteriorized heart was attached to an artificial perfusion system (modified Langendorff apparatus) used for normothermic perfusion. Isolated hearts were stabilized with normothermic perfusion with a constant flow (7 mL/min) for 20 minutes delivered by a microtubing pump (MP2; Analytical West, Inc., Corona, CA, USA). The hearts in the control group were perfused with a constant flow (10 mL/min) derived by a microtubing pump (MP2) delivering Krebs–Henseleit solution aerated with a gas mixture of 95% oxygen and 5% carbon dioxide at 37.5°C. Hearts in the experimental group were perfused as in the control group for the first half (10 minutes) of the preischemic phase, whereas for the second 10 minutes iloprost was added to the Krebs–Henseleit solution (0.45 µg/kg per hour). A 3-0 silk was placed at the cardiac apex and attached to isometric tension transducers of a GRASS polygraph (Model 7D; Grass Instrument Company, Quincy, MA, USA) used to record heart rate and contractility levels. After stabilization, all hearts were subjected to ischemia for 20 minutes. Following ischemia, the hearts underwent reperfusion for 20 minutes with the Krebs–Henseleit solution.

Heart rate (pulse/min), aortic pressure (mmHg), and contractility (mm) were measured at 3 time points (preischemia, postischemia, and reperfusion). Samples of perfusate and tissue were taken during the same 3 time points. The samples were then frozen and stored at –70°C for subsequent malondialdehyde (MDA), glutathione, and nitric oxide (NO) level analyses.

Iloprost was obtained as Ilomedin® (20 µg/mL, Bayer AG Leverkusen, Germany), Krebs–Henseleit solution was prepared by appropriate composition of given compounds. Spectrophotometric analysis was performed by using a Bausch & Lomb Spectronic 21 device (Bausch + Lomb, Bridgewater,

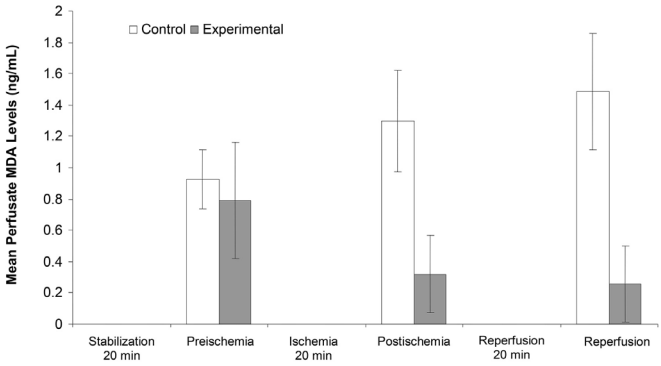


Figure 6. Mean perfusate MDA values of control and experimental groups (x ± SD).

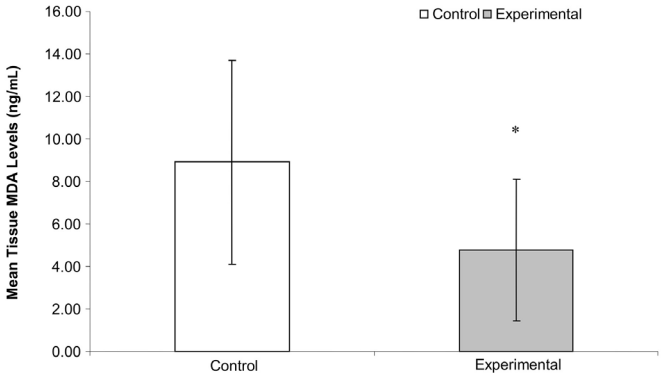


Figure 7. Mean tissue MDA values of control and experimental groups (x ± SD). *$P$ <.05: Compared to control group.

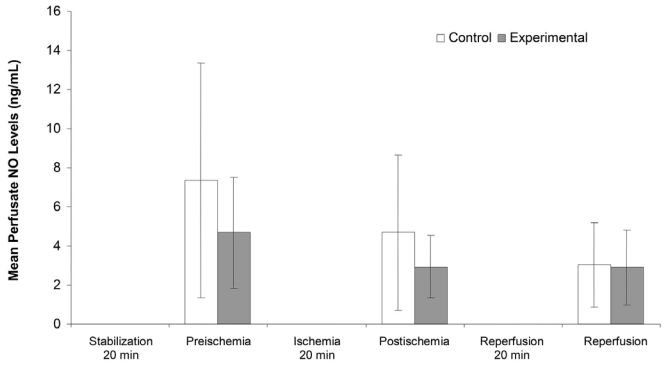


Figure 8. Mean perfusate NO values of control and experimental groups (x ± SD).

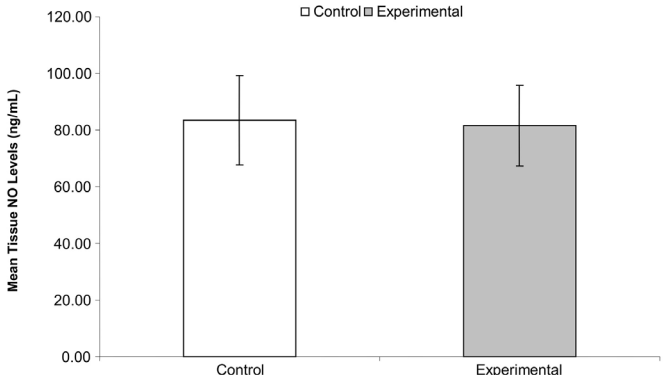


Figure 9. Mean tissue NO values of control and experimental groups (x ± SD).

NJ, USA). The Kurtel method was used for glutathione and MDA measurements, and Griess reaction was used for NO measurements in perfusate samples [Kurtel 1992]. Data were analyzed by using SSPS version 13 (Chicago, IL, USA). Differences between intragroups and intergroups were analyzed with the Mann–Whitney U test. $P$ <.05 was the accepted level of significance.

## RESULTS

Heart rate and aortic pressure levels of preischemic, postischemic, and reperfusion periods did not change statistically among time periods and were not statistically different between control and experimental hearts (Figures 1-2). At the same time, myocardial contractility was found to be significantly higher in the experimental group as compared to the control group in all 3 time periods ($P$ <.001). Additionally, the highest contractility values were obtained during the reperfusion period (Figure 3).

In terms of measures of myocardial tissue injury, postischemic and reperfusion perfusate levels of glutathione were found to significantly decrease as compared to preischemic levels in the control group. Experimental subgroups of preischemic, postischemic, and reperfusion periods were observed to have statistically similar values for perfusate glutathione levels. Tissue glutathione levels were significantly higher in the experimental group as compared to levels of the controls (Figures 4-5).

Postischemic and reperfusion levels of MDA were significantly higher compared to the preischemic period in the control group. However, there were no significant changes of MDA levels among preischemic, postischemic, and reperfusion periods within the experimental group (Figure 6).Tissue MDA levels were also found to be significantly lower in the experimental group than those in the control group (Figure 7).

Perfusate NO levels were observed to be significantly lower in the postischemic and reperfusion periods compared to the preischemic period in both the experimental and control groups (Figure 8). There was no statistical difference in tissue NO levels between subgroups or time periods (Figure 9).

## DISCUSSION

The necessary treatment for myocardial ischemia is reperfusion, to be performed as quickly as possible. However, even with reperfusion, myocardial injury may occur. As such, it is necessary to develop additives to perfusion that can protect tissue from ischemia/reperfusion. It is also

of particular importance to avoid tissue ischemia beyond 20 minutes because this can cause irreversible damage [Nakanishi 1997]. In this study, iloprost (0.45 µg/kg per hour), added to the Krebs–Henseleit solutions for the last half of the 20-minute ischemic phase, was found to have a significant relieving effect on myocardial ischemia-reperfusion injury in isolated guinea pig hearts perfused with this solution.

Improved cardiac output secondary to an increase in myocardial contractility seems to be the most prominent therapeutic effect of iloprost. It has been stated that iloprost has the ability to decrease myocardial oxygen consumption by lowering blood pressure while at the same time avoiding an increase in heart rate [Swedberg 1987]. In this study, iloprost was able to maintain cardiac function and improve contractility.

Reperfusion injury following ischemia is caused by free radical release initiated by the oxidative burst during reperfusion period. This burst leads to myocardial contractile dysfunction in the reperfusion period. In experimental models, it is difficult to measure levels of free oxygen. Therefore, in this study, we monitored the levels of markers of injury from radicals that are more easily measured than the radicals themselves. Because of the instability of free radicals at neutral pH values, their biological activity is difficult to measure at tissue level. We measured levels of MDA, glutathione, and NO at 3 different time points to assess the change in levels of free radical activity.

MDA was chosen as an index of free radical actvity because it is the end product of the lipid peroxidation reaction, which is specific to unsaturated fatty acids and free radicals [Sinclair 1990]. In the control group, postischemic and reperfusion period levels of MDA were significantly higher as compared to MDA levels in the preischemic period of the control group. Additionally, there were no significant changes in MDA levels between preischemic, postischemic, and reperfusion periods in the experimental group. We propose that iloprost in the perfusates of the experimental group prevented the MDA increase by suppressing the lipid peroxidation reaction. Tissue MDA levels in the experimental group were also found to be significantly lower than those in the control group. Iloprost therapy, as shown in the experimental group in our study, can protect the heart from ischemia by decreasing lipid peroxidation.

The second indicator of reperfusion injury that we monitored was glutathione, an endogenous substance activated during reperfusion injury in order to neutralize the negative effects of reactive oxygen intermediates. Because endogenous cardiac glutathione is a protective agent against short-term myocardial ischemia, healing from ischemia-reperfusion injury is limited by glutathione replacement [Blaustein 1989]. Glutathione levels should decrease with greater tissue injury. In this study, postischemic and reperfusion perfusate levels of glutathione in the experimental group were found to decrease markedly as compared to preischemic levels, supporting the protective role of iloprost in ischemic conditions. Experimental subgroups of preischemic, postischemic, and reperfusion periods were observed to have similar values for perfusate glutathione levels. Tissue glutathione levels were also higher in the experimental group when compared to those in the controls. These findings were interpreted as decreased glutathione need in the iloprost-treated group because of protection of the myocardium against ischemia.

Finally, the third ischemia-reperfusion injury marker that we measured was NO. NO is known to act as a vasodilator, an inhibitor of thrombocyte aggregation, an antioxidant, and a neutrophil antiadhesive molecule. NO release is reduced in myocardial ischemia-reperfusion injury [Ma 1993]. In our study, perfusate NO levels were observed to decrease in the postischemic and reperfusion periods as compared to the preischemic period in both experimental and control groups as a natural result of the ischemic insult. Despite possessing NO-like effects, in our study, iloprost had no remarkable effect on perfusate and tissue NO levels.

There have been many experimental studies with various substances and techniques on prevention of myocardial ischemia-reperfusion injury as a prognostic risk factor; myocardial ischemia-reperfusion injury is frequently encountered during open heart surgery [Ambrosio 1992; Pucheu 1993; Qiu 1995; Ding 1996; Knezl 1999; Cui 2003; Flynn 2003; Saeed 2005; Turan 2005]. In this study, we aimed to clarify and confirm the effects of iloprost on myocardial ischemia-reperfusion injury, by analyzing the levels of MDA, glutathione, and NO in tissue and perfusates. We conclude that iloprost has significant protective effects against myocardial ischemia-reperfusion injury, and we support this conclusion by our findings in the differences in tissue and perfusate levels of glutathione and MDA. Still, further studies are necessary to provide more detailed explanations for the mechanism of the effects of iloprost.

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## REFERENCES

Ambrosio G, Villari B, Chiariello M. 1992. Calcium antagonists and experimental myocardial ischemia reperfusion injury. J Cardiovasc Pharmacol 20(suppl 7):S26-9.

Blaustein A, Deneke SM, Stolz RI, Baxter D, Healey N, Fanburg BL. 1989. Myocardial glutathione depletion impairs recovery after short periods of ischemia. Circulation 80(5):1449-57.

Cui J, Das DK, Bertelli A, Tosaki A. 2003. Effects of L-carnitine and its derivatives on postischemic cardiac function, ventricular fibrillation and necrotic and apoptotic cardiomyocyte death in isolated rat hearts. Mol Cell Biochem 254(1-2):227-34.

Das DK, Engelman RM, Rousou JA, Breyer RH, Otani H, Lemeshow S. 1986. Pathophysiology of superoxide radical as potential mediator of reperfusion injury in pig heart. Basic Res Cardiol 81(2):155-66.

Ding YF, Li YL, Ho SY. 1996. [Ischemic preconditioning and exogenous L-arginine reduce infarct size in rabbit heart]. Sheng Li Xue Bao 48(6):564-70. Chinese.

Edmunds LH Jr. 2002. The evolution of cardiopulmonary bypass: lessons to be learned. Perfusion 17(4):243-51.

Flynn JD, Akers WS. 2003. Effects of the angiotensin II subtype 1 receptor antagonist losartan on functional recovery of ısolated rat hearts undergoing global myocardial ıschemia-reperfusion. Pharmacotherapy 23(11):1401-10.

Forman MB, Virmani R, Puett DW. 1990. Mechanisms and therapy of myocardial reperfusion injury. Circulation 81(3 suppl):IV69-78.

Gardner TJ, Stewart JR, Casale AS, Downey JM, Chambers DE. 1983. Reduction of myocardial ischemic injury with oxygen-derived free radical scavengers. Surgery 94(3):423-7.

Knezl V, Sotniková R, Okruhlicová L, Navarová J. 1999. Effect of stobadine on cardiac injury induced by ischemia and reperfusion. Life Sci 65(18-19):1931-3.

Kurtel H, Granger DN, Tso P, Grisham MB. 1992. Vulnerability of intestinal interstitial fluid to oxidant stress. Am J Physiol 263(4 Pt 1):G573-8.

Ma XL, Weyrich AS, Lefer DJ, Lefer AM. 1993. Diminshed basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. Circ Res 72(2):403-12.

Nakanishi K, Inoue M, Sugawara E, Sano S. 1997. Ischemic and reperfusion injury of cyanotic myocardium in chronic hypoxic rat model: changes in cyanotic myocardial antioxidant system. J Thorac Cardiovasc Surg 114(6):1088-96.

Pucheu S, Coudray C, Tresallet N, Favier A, de Leiris J. 1993. Effect of iron overload in the isolated ischemic and reperfused rat heart. Cardiovasc Drugs Ther 7(4):701-11.

Qiu Y, Galiñanes M, Hearse DJ. 1995. Protective effect of nicorandil as an additive to the solution for continous warm cardioplegia. J Thorac Cardiovasc Surg 110(4 Pt 1):1063-72.

Saeed SA, Waqar MA, Zubairi AJ, et al. 2005. Myocardial ischaemia and reperfusion injury: reactive oxygen species and the role of neutrophil. J Coll Physicians Surg Pak 15(8):507-14.

Simpson PJ, Mickelson J, Fantone JC, Gallagher KP, Lucchesi BR. 1987. Iloprost inhibits neutrophil function in vitro and in vivo and limits experimental infarct size in canine heart. Circ Res 60(5):666-73.

Sinclair AJ, Barnett AH, Lunec J. 1990. Free radicals and antioxidant systems in health and disease. Br J Hosp Med 43(5):334-44.

Swedberg K, Hed P, Wadenvik H, Kutti J. 1987. Central haemodynamic and antiplatelet effects of iloprost--a new prostacyclin analogue--in acute myocardial infarction in man. Eur Heart J 8(4):362-8.

Turan B, Saini HK, Zhang M, Prajapati D, Elimban V, Dhalla NS. 2005. Selenium improves cardiac function by attenuating the activation of NF-κB due to ischemia–reperfusion ınjury. Antioxid Redox Signal 7(9-10):1388-97.