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***Experimental Study** / Deneysel Çalışma*

Levosimendan attenuates ischemia/reperfusion injury of the heart better than ischemic preconditioning during moderate hypothermia

Levosimendan kalbin iskemi/reperfüzyon hasarında orta dereceli hipotermide iskemik önkoşullamadan daha iyi koruma sağlamaktadır

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Background: This study aims to demonstrate whether levosimendan offers additive cardioprotection compared with ischemic preconditioning during moderate hypothermia.

Methods: Thirty-six male Wistar rats were selected for the study. Rats' hearts were rapidly excised after application of ketamine. Aorta was cannulated and infusion of 37 °C Krebs-Henseleit Buffer (KHB) was started to provide heart stabilization for 10 minutes. Then, the isolated hearts were randomly assigned to one of six groups: group 1 (normothermia 37 °C-control), group 2 (hypothermia 28 °C-Control), group 3 (normothermia-ischemic preconditioning), group 4 (hypothermia-ischemic preconditioning), group 5 (normothermia-levosimendan), group 6 (hipothermi-levosimendan). Levosimendan was added to KHB solution (24 µg/kg for 10 minutes loading, and 0.1 µg/kg/minute for maintenance). Data were analyzed by using Kruskal Wallis and Mann-Whitney U test, and *p* values of <0.05 and <0.008 were accepted significant after using Bonferroni adjustment.

Results: The tissue malondialdehyde (MDA) levels in group 4 were significantly increased compared to group 6 (median range 1.85 vs. 0.70, respectively; p=0.004). Sodium, potassium adenosine triphosphatase (Na^+-K^+ ATPase) enzyme activity was significantly protected in group 6 compared to group 4 (303.6 vs. 209.1, respectively; p=0.004). Group 4 revealed extensive TUNEL-positive cardiomyocytes compared to group 6. Percentage of apoptotic cell staining was 37.5% and 10%, respectively (p=0.004). Electron microscopic evaluation of group 6 showed normal morphological composition characterized by regular arrangement of myofibrils and protected mitochondrial structure and sarcomer in comparison with other groups.

Conclusion: Results of this study showed that separate use of levosimendan and hypothermia provided protective effects on ischemic myocardium. However, pretreatment with levosimendan provided better protection under the moderate hypothermic condition.

Keywords: Heart; hypothermia; ischemic preconditioning; levosimendan.

Amaç: Bu çalışmada levosimendanın orta dereceli hipotermi sırasında iskemik önkoşullamaya kıyasla ek kardiyak koruma sağlayıp sağlamadığı araştırıldı.

Çalışma planı: Çalışma için 36 erkek Wistar sıçan seçildi. Ketamin uygulandıktan sonra sıçan kalpleri hızlıca çıkarıldı. Aort kanüle edildi ve kalp stabilizasyonunu sağlamak için 10 dakika 37 °C Krebs-Henseleit Buffer (KHB) infüzyonu başlatıldı. Daha sonra, izole edilen kalpler randomize olarak altı gruba ayrıldı: grup 1 (normotermi 37 °C-kontrol), grup 2 (hipotermi 28 °C-kontrol), grup 3 (normotermi-iskemik önkoşullama), grup 4 (hipotermi-iskemik önkoşullama), grup 5 (normotermi-levosimendan), grup 6 (hipotermi-levosimendan). Levosimendan KHB solüsyonuna eklendi (10 dakika yükleme için 24 µg/kg, idame için 0.1 µg/kg/dakika). Veriler Kruskal Wallis ve Mann-Whitney U testi kullanılarak analiz edildi ve Bonferroni düzeltmesi kullanıldıktan sonra p<0.05 ve p<0.008 değerleri anlamlı kabul edildi.

Bulgular: Doku malondialdehid (MDA) seviyeleri grup 6'ya kıyasla grup 4'te anlamlı olarak arttı (sırasıyla, ort. dağılım 1.85'e kıyasla 0.70; p=0.004). Sodyum, potasyum adenozin trifosfataz (Na^+-K^+ ATPase) enzim aktivitesi grup 4'e kıyasla grup 6'da anlamlı şekilde korundu (sırasıyla 303.6'ya kıyasla 209.1; p=0.004). Grup 4, grup 6'ya kıyasla yoğun TUNEL pozitif kardiyomiyosit gösterdi. Apoptotik hücre boyanma yüzdesi sırasıyla %37.5 ve %10 idi (p=0.004). Diğer gruplara göre, grup 6'nın elektron mikroskopik incelemesi düzenli miyofibriler ve korunmuş mitokondriyal yapı ve sarkomer ile karakterize normal morfolojik kompozisyon gösterdi.

Sonuç: Bu çalışmanın sonuçları, levosimendan ve hipoterminin ayrı ayrı kullanımının iskemik miyokard üzerinde koruyucu etki sağladığını göstermiştir. Öte yandan, levosimendan ile ön tedavi orta dereceli hipotermi durumunda daha iyi koruma sağlamıştır.

Anahtar sözcükler: Kalp, hipotermi, iskemik önkoşullama, levosimendan.

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Portions of this study have been presented in abstract form at the Annual Symposium of EACTA Meeting (June 11-14, 2008, Antalya, Turkey).

The activation of mitochondrial adenosine triphosphate-dependent potassium (mitoK_{ATP}) channels in cardiac myocytes is a potent cardioprotective mechanism, and the increased potassium influx associated with the mitoK_{ATP} channel opening preserves the mitochondrial function in situations of ischemia and/or reperfusion.[1] A number of studies have suggested that opening the K_{ATP} channels either by ischemia, or hypoxia[2] or by selective potassium channel opener drugs,[3] exerts a protective effect on the ischemic-reperfused heart, and this is the final common step that underlies all precondition like states, including those elicited by ischemic and pharmacologically-induced preconditioning. Ischemic preconditioning is defined as an endogenous mechanism by which a brief period of ischemia and reperfusion attenuate myocardial tolerance against the adverse effects of a subsequent prolonged period of ischemia.[4]

Levosimendan is a novel inotropic agent used in the management of acute decompensated heart failure which mediates the cardiac effect via the calcium sensitization of the contractile proteins. It has also been suggested that this drug protects the ischemic myocardium and that it decreases the infarct size in coronary-ligated animals.[5] Levosimendan may even exert anti-apoptotic properties linked to the activation of mitoK_{ATP} channels,[6] and it also exerts vasodilatory effects through the opening of K_{ATP} in vascular smooth muscle cells.[7]

To date, most studies have demonstrated the advantage of levosimendan as an inotrope on the mechanical recovery of the heart and as a cardioprotective agent on the myocardium or other organs in normothermia,[5,8] but the benefits of this drug with regard to hypothermic ischemia-reperfusion (I/R) have only been shown in small clinical and animal studies.[9] However, the current knowledge about preconditioning during hypothermic ischemia does not allow for medical professionals to predict the extent of the injury in the myocardium because the molecular mechanisms of preconditioning vary at different temperatures.[10]

In a recent study, we showed that treatment with levosimendan preserved the myocardial ultrastructure and enzymatic membrane activity in isolated rat hearts that were in deep hypothermic cardioplegic arrest.[11] As a follow-up, we conducted this study to evaluate whether levosimendan affords additive cardioprotection compared with ischemic preconditioning during moderate hypothermia as they pertain to myocardial apoptosis and the degree of mitochondrial degeneration.

MATERIALS AND METHODS

All experimental procedures and protocols used in this investigation were reviewed and approved by the local ethics committee that oversees animal experiments, and the animal care experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No: 85-23, revised 1996). In this study, 36 Wistar rats (250-300 g), who were randomly divided into six groups composed of six rats each, fasted overnight but were allowed free access to water. In addition, the surgery took place in a room kept at 24 °C.

Levosimendan (Simdax®, Orion Corporation, Espoo, Finland) was diluted appropriately and added to a Krebs-Henseleit Buffer (KHB) solution that was used for stabilization in the perfusion period in the treatment groups.

The rats were anesthetized with intramuscular ketamine 60 mg/kg^{-1} and systemically heparinized by injecting 3.5 mg (3500 IU) of heparin into the peritoneal cavity. They were then operated on when they were unresponsive to noxious stimulation. A midline sternotomy was performed, and each heart was rapidly excised and immersed in the 37 °C KHB solution. Next, the aorta was cannulated distal to the aortic valve, and the hearts were mounted on a modified Langendorff apparatus. Coronary circulation was started by retrograde aortic perfusion using the KHB solution [Constituents in mmol l^{-1}: sodium chloride (NaCl) 118, calcium chloride ($CaCl_2$) 3.0, potassium chloride (KCl) 4.7, monopotassium phosphate (KH_2PO_4) 1.2, sodium bicarbonate ($NaHCO_3$) 25, magnesium sulfate ($MgSO_4$) 1.2, and glucose 11.1] at 37 °C for a 10 minute stabilization period while perfusion pressure in the aorta was kept constant at a pressure of 50 mmHg in all of the groups. This aortic pressure was obtained through a side arm of the cannula that was connected to a pressure transducer. After the period of stabilization, the hearts were randomly assigned to one of the six groups.

In the normothermic control group (group 1), the hearts were perfused with KHB for 30 minutes and then kept at 37 °C in an isotonic saline (0.9% NaCl)-jacketed heart chamber for 30 minutes of global ischemia followed by 30 minutes of reperfusion at 37 °C.

In the hypothermic control group (group 2), the rats underwent the same procedure as group 1 except that the hearts were kept at 28 °C followed by 30 minutes of reperfusion at 37 °C.

In the normothermic-ischemic preconditioning group (group 3), the hearts underwent two five-minute cycles of global ischemia followed by 10 minute washout periods before 30 minutes of global ischemia.

In the hypothermic-ischemic preconditioning group (group 4), the animals underwent the same procedure as group 3 except that these rats underwent 30 additional minutes of reperfusion at 28 °C followed by 30 minutes of reperfusion at 37 °C.

In the normothermic-levesimendan group (group 5), the levosimendan was added to the KHB (24 μg kg^{-1} for loading over 10 minutes and 0.1 μg kg^{-1} min^{-1} for an infusion of 20 minutes) and then infused. This was followed by 30 minutes of ischemia at 37 °C.

In the hyporthermic-levosimendan group (group 6), the rats underwent the same procedure as group 5, but the ischemia occurred at 28 °C instead of 37 °C. This was followed by 30 minutes of reperfusion using drug-free KHB at 37 °C.

The experiment protocol is summarized in Figure 1. At the end of the reperfusion period, the hearts were homogenously divided for biochemical assays, and a histological analysis was carried out using electron microscopy. In addition, a histopathological analysis was also conducted.

The malondialdehyde (MDA) levels, as an index of lipid peroxidation, were determined by a thiobarbituric acid (TBA) reaction utilizing the method of Yagi.[12] That method depends on the amount of pink color produced by the interaction of the TBA with the MDA as a result of lipid peroxidation, and we chose the colored reaction 1,1,3,3-tetrathoxypropane as our

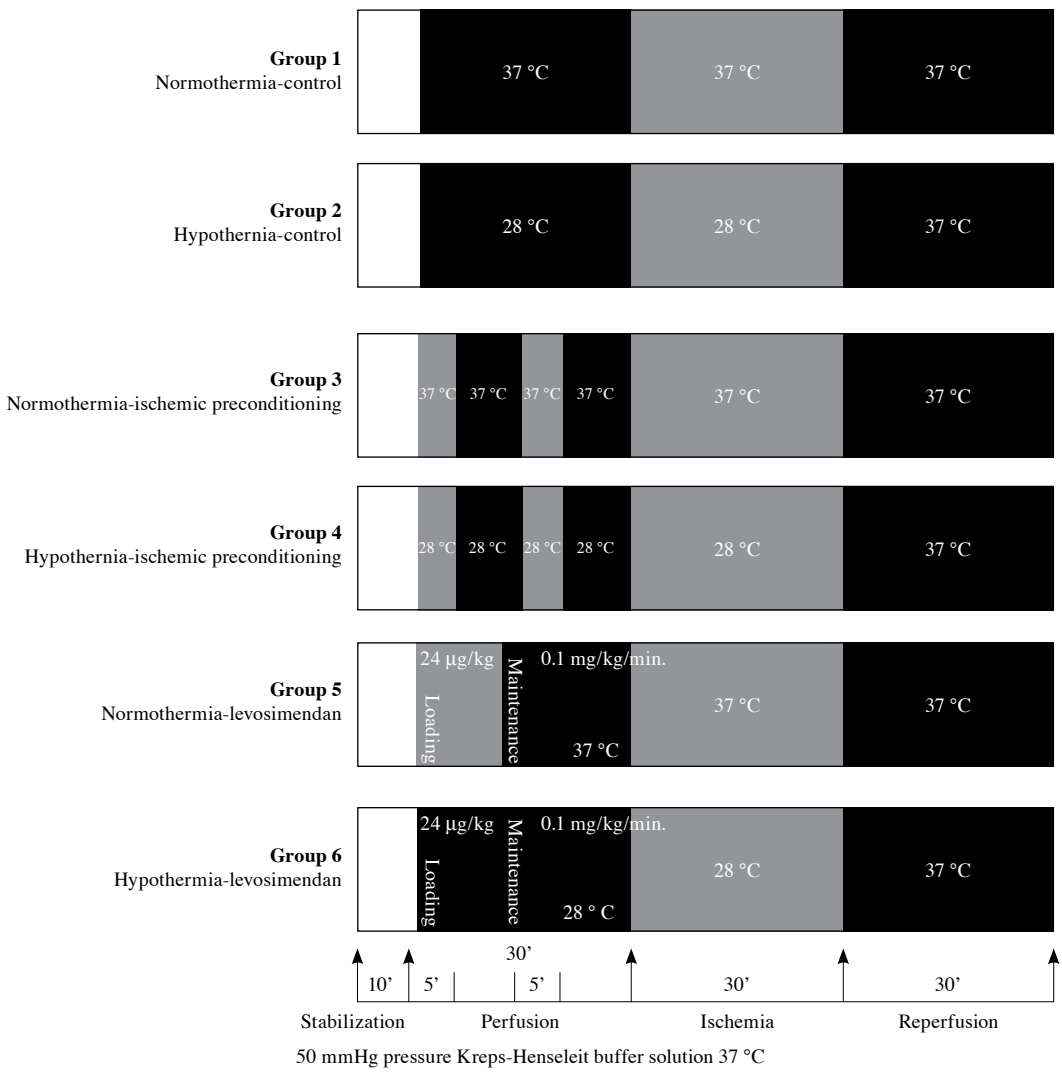

Figure 1. Experiment protocol.

primary standard. The MDA levels in the heart tissue were expressed as nmol gr^{-1}.

Ten percent of the homogenates of the tissue were prepared in 0.3 M sucrose, which contained 1 mM magnesium, and homogenized for 90 seconds using a Teflon pestle clearance of 0.25-0.38 mm at 1000 rpm/min. To remove the debris, it was then centrifuged at 1000 rpm/min for 15 minutes. ATPase activities were determined on the resulting supernatants by measuring rate of liberation of inorganic phosphate (Pi) from disodium ATP incubation media were made up as described previously.[13] The adenosine 5' triphosphatases were as follows: Na^+-K^+ ATPase (mM)-MgCl2 6, KCl 5, NaCl 100, ethylenediaminetetraacetic acid (EDTA) 0.1, and tris(hydroxymethyl) aminomethane hydrochloride (tris-HCl) buffer pH 7.4, 135. Enzyme activities were calculated as nmol Pi^{-1} h^{-1} protein. The protein content was determined according to the method described by Lowry using bovine serum albumin the standard.[14]

The terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining method

The myocardium was stored in a 10% formaldehyde solution at 20 °C. Afterwards, the formaldehyde was fixated into routine paraffin blocks, and paraffin sections measuring 5 µm in thickness were then prepared. To investigate DNA fragmentation at the myocytes via the TUNEL method, we selected the In Situ Apoptosis Detection Kit (BioGen Medikal Aletler Tic. Ltd. Sti., Istanbul, Turkey). After the deparaffined and rehydrated sections were pretreated with proteinase K for 15 minutes at room temperature, the endogen peroxidase activity was quenched with 2% hydrogen peroxide (H_2O_2). The slices were then incubated at 37 °C for 60 minutes in a moist chamber with 50 µl of terminal deoxynucleotidyl transferase (TdT) buffer. Finally, the reaction was visualized using a streptavidin-biotin-peroxydase complex and diaminobenzidine (DAB), and the TUNEL-labeled slides were counterstained with 1% methyl green. The apoptotic cells were then counted under a light microscope, and the results were expressed as a percentage (%).

Ultrastructural Study [Transmission Electron Microscopy (TEM)]

For the TEM evaluation, the samples were fixed with 2.5% gluteraldehyde that was post-fixed with 1% osmium tetroxide and dehydrated in graded alcohol. They were then cleared with propylene oxide and embedded in epoxy resin. Next, thin sections (50-70 nm) were cut by the Leica UCT-125 ultramicrotome (Leica Microsystems GmbH, Wien, Austria) and contrasted with uranyl acetate and lead citrate. Afterwards, the sections were examined and photographed utilizing the JEOL JEM-1011 TEM (JEOL, Ltd., Tokyo, Japan) by a histologist blinded to the study groups. The degree of mitochondrial degeneration was determined from 10 randomized fields per section, and 100 mitochondria per sample were graded at a magnification of 10,000x. All of the mitochondria were counted in all fields by commercially available software, and the mitochondrial damage was scored by assigning a numerical value according to the degree of morphological alterations using the following four grades: Grade 1: Normal mitochondria; Grade 2: Decay in cristae organization; Grade 3: Loss of cristae and mitochondrial swelling; Grade 4: Mitochondria totally broken (Figure 2).

Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics for Windows version 19.0 software package (IBM Corporation, Armonk, NY, USA), which was used with the permission and license of Bulent Ecevit University. The variables were expressed as medians, and the data was analyzed using the Kruskal-Wallis test. In addition, post-hoc comparisons were performed using the Mann-Whitney U test. A value of $p<0.05$ was considered to be statistically significant for the analyses of variance (ANOVA) and a value of $p<0.008$ was accepted as having significance for the Mann-Whitney U test when the Bonferroni adjustment was applied.

RESULTS

The tissue MDA levels in groups 1 and 2 were significantly increased compared with the other

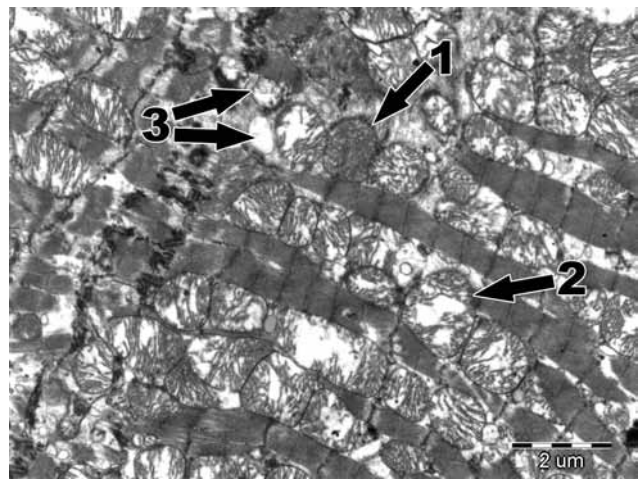

Figure 2. Scoring of the mitochondria (TEM x 10,000). Grade 1: Normal mitochondria; Grade 2: Decayed cristae organization; Grade 3: Loss of cristae and mitochondrial swelling; Grade 4: Mitochondria totally broken.

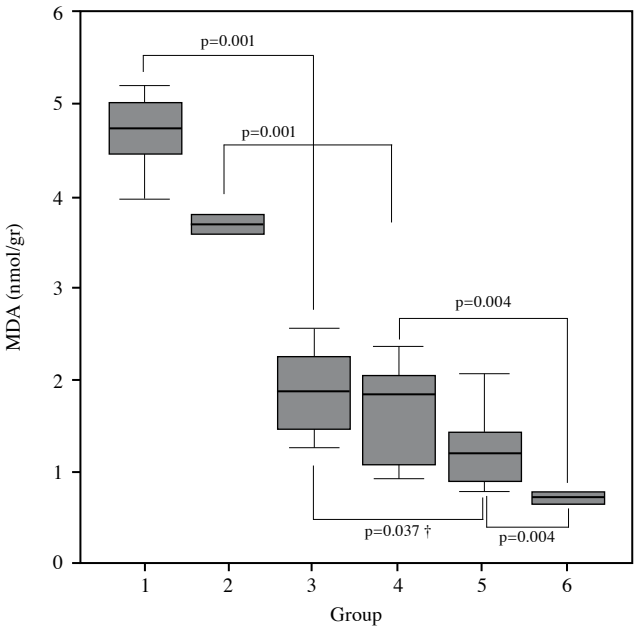

Figure 3. Distribution of tissue malondialdehyde levels. MDA: Malondialdehyde; † Not significant according to the Bonferroni correction.

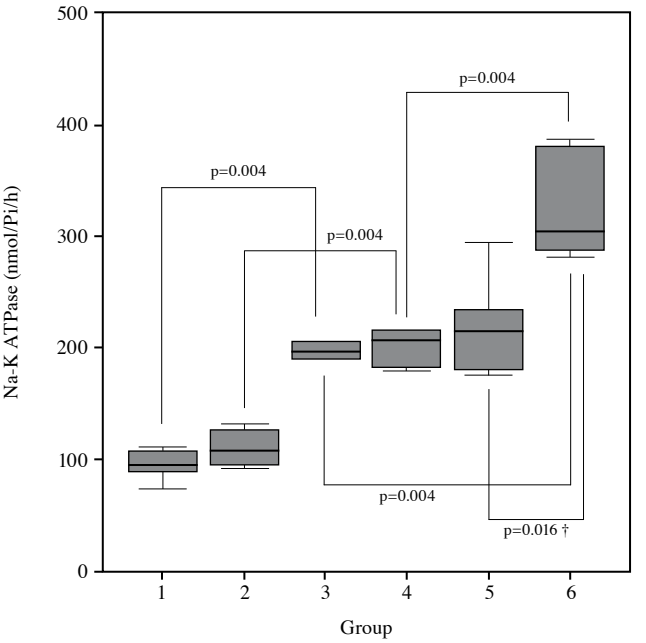

Figure 4. Distribution of Na^+-K^+ ATPase levels. † Not significant according to the Bonferroni correction.

study groups (Figure 3). The levels were also significantly higher in group 3 than in group 5 (median 1.90 vs. 1.25, respectively; p=0.037), and they also differed between groups 4 and 6 (median 1.85 vs. 0.70, respectively; p=0.004) and 5 and 6 (median 1.25 vs. 0.70, respectively; p=0.004) (Figure 3).

The tissue Na^+-K^+ ATPase enzyme activity of groups 1 and 2 were significantly decreased compared with other study groups (Figure 4). Furthermore, the enzyme activity in group 3 was also markedly decreased compared with group 6 (198.1 vs. 303.6, respectively; p=0.004), but group 6 was significantly more protected than group 4 (303.6 vs. 209.1, respectively; p=0.004). Additionally, a comparison between groups 5 and 6 showed better protection in group 6 (214.0 vs. 303.6; p=0.016).

Groups 1 and 2 had more apoptotic cells than the other study groups, and group 4 revealed extensive TUNEL-positive cardiomyocytes (Figure 5a) compared with group 6 (Figure 5b) (37.5% vs. 10%, respectively; p=0.004). Similarly, more apoptotic

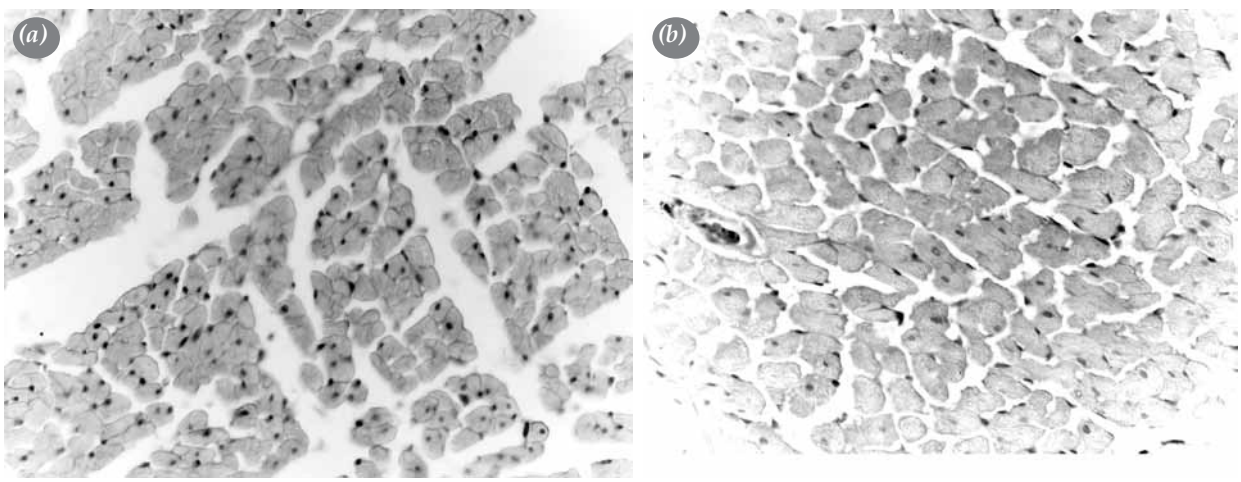

Figure 5. (a) Diffuse terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells in group 4; Brown stained nuclei of apoptotic cardiomyocytes (SEMx10.000). **(b)** Sparse staining of apoptotic cells (SEMx10.000).

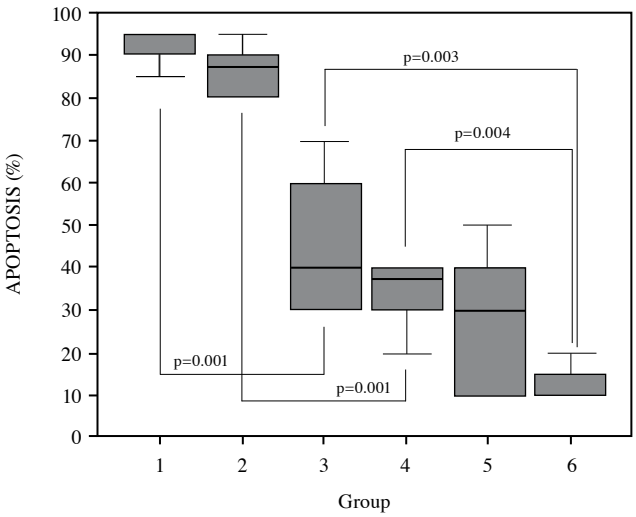

Figure 6. Number of apoptotic cells shown as percentages.

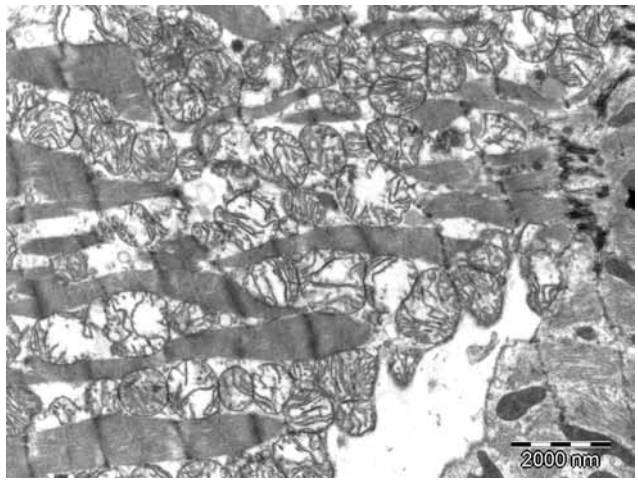

Figure 7. Loss of normal architecture of some cardiac muscle fibers with irregularity along with the accompanying myofibril thinning and breakage TEM x 10,000).

cells were found in group 3 than in group 6 (40% vs. 10%, respectively; p=0.003), but there were no statistically significant differences between the other groups (Figure 6).

The cardiac muscle fibers of the left ventricle in groups 1 and 2 showed a loss of normal architecture, with some cardiac muscle fibers showing irregularity along with myofibril thinning and breakage (Figure 7). Furthermore, degenerative changes in the mitochondria and intercalated discs were also seen in these two control groups. Groups 3 and 4 revealed myofibrillar derangement and degeneration as well as swollen mitochondria with thickened, disoriented, and disintegrated cristae; however, the fibers in these two groups were observed to be in better condition that those in groups 1 and 2 (Figure 8). In addition, the TEM results were better for groups 3 and 4 than for group 5, and group 6 showed normal morphological composition characterized by the regular arrangement of myofibrils and protected structure of the sarcomere and mitocondria (Figure 9). A semi-quantitative grading of mitochondrial injuries showed a statistical difference between groups 4 and 6, especially at grades 1 and 2 (Table 1).

DISCUSSION

This experimental study revealed that levosimendan and hypothermia provide protective effects on the

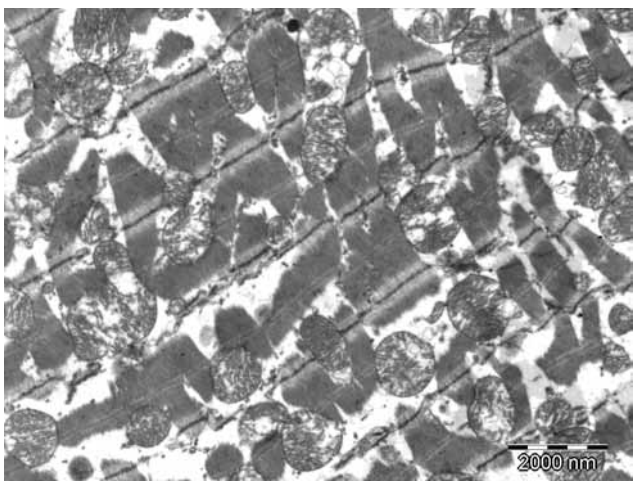

Figure 8. Myofibrillar derangement and degeneration, swollen mitochondria with thickened, disoriented cristae (transmission electron microscopy x 10.000).

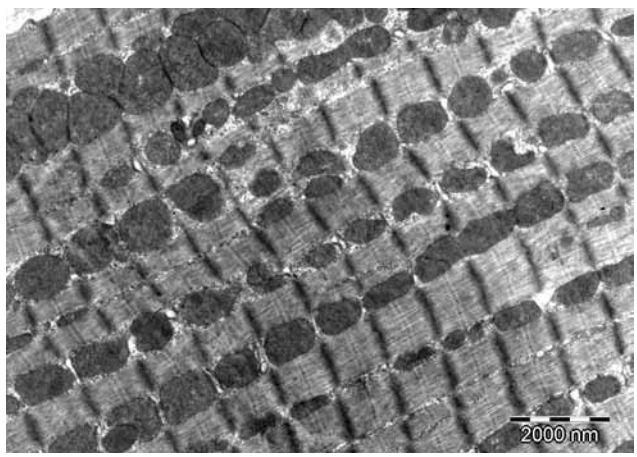

Figure 9. Normal morphological composition characterized by regular arrangement of myofibrils and protected structure of sarcomere and mitochondria (transmission electron microscopy x 10.000).

Table 1. Semi-quantitative grading of injuries obtained after examining more than 2,000 mitochondria in groups 4 and 6

	Group 4	Group 6	
	Mean±SD	Mean±SD	*p*
Grade 1	88.4±1.8	97.0±0.9	0.002
Grade 2	10.9±1.9	2.3±0.9	0.002
Grade 3	0.7±0.7	0.4±0.5	0.589
Grade 4	00±00	00±00	–

SD: Standard deviation; group 6 showed a much larger proportion of preserved mitochondria (grade 1) than group 4; group 4 showed that the proportion of injured mitochondria (grade 2) was significantly higher than in group 6.

ischemic myocardium, and these effects are additive. Pretreatment with levosimendan provided better protection in our study groups, especially under moderate hypothermic (28 °C) conditions, compared with IP with respect to the cell wall enyzmes, myocardial apoptosis, and degree of mitochondrial degeneration.

Furthermore, we determined that myocardial hypothermia was an effective method for protecting the myocardium because it lowered the myocardial metabolism during ischemia. Hypothermic protection of the ischemic myocardium is associated with the preservation of high-energy phosphates, which may facilitate the maintenance of membrane integrity during ischemia.[15] However, hypothermia alone did not offer satisfactory myocardial protection in our study. Therefore, the quest for additional protective methods must continue, with one possibility being myocardial conditioning, which has been the focus of multiple randomized trials since 1986.[16]

Pharmacological conditioning with levosimendan and volatile anesthetics has significantly improved cardiac surgical outcomes in randomized trials, large observational trials, and meta-analyses,[17] whereas ischemic myocardial conditioning has consistently protected the heart during ischemia and reperfusion both in the catheterization laboratory and the operating room.[18]

In addition, IP has shown clinical benefits in cardiac surgery. Walsh et al.[19] concluded that it may provide additional myocardial protection over cardioplegia alone and expressed the need for a large randomized controlled clinical trial to further investigate this hypothesis.

The primary targets of reactive oxygen species (ROS) attacks are the polyunsaturated fatty acids in the membrane lipids since they cause lipid peroxidation that can lead to disorganized cell structure and function. Malondialdehyde is the byproduct of a breakdown in the major chain reactions, which can lead to significant oxidation of polyunsaturated fatty acids, such as linoleic and linolenic acids. Thus, it is able to serve as a reliable marker of oxidative stress (OS).[20] In this study, the MDA levels were clearly decreased in group 6 compared with groups 4 and 5, suggesting that levosimendan alone is effective in the prevention of lipid peroxidation. However, better results occur when it is used in combination with hypothermia. On the other hand, the results in the normothermic IP groups (3 and 4) in this study were not as good as those for group 5, the normothermic-levosimendan group.

The membrane-bound enzyme Na-K ATPase executes cellular functions in ionic and osmotic balance as well as in active transport. Moreover, lipid peroxidation changes the membrane fluidity and enzyme activity. We determined that the enzyme activity of Na^+-K^+ ATPase was significantly higher in group 6 than in groups 3, 4, or 5 and that these higher levels of ATPase activity might have protected the myocardium from more severe injury following I/R. This protective effect of levosimendan was also found in our previous study.[11]

Apoptosis, or programmed cell death, is the highly conserved and physiological process of eliminating cells in multicellular organisms.[21] Cells that undergo apoptotic death present a typical morphology consisting of cell shrinkage and nuclear fragmentation. This can be quantified in histopathological tissue sections by TUNEL staining.[22] In this study, the percentage of apoptosis was the lowest in group 6, and this protective effect was statistically superior to what we found in the other groups. Similar findings were reported by Maytin and Colucci[23] who showed in their *in vitro* study that levosimendan, even at very low concentrations, protected cardiomyocytes from H_2O_2-induced apoptosis by activating mitochondrial ATP-dependent K^+ channels. Öztürk et al.[24] also found that levosimendan can induce B-cell lymphoma (Bcl-2) expression and reduce the number of TUNEL-positive cardiomyocytes in isolated rat hearts. They also showed that the myocardial infarct size was reduced compared with the controls.

The protection offered by levosimendan as well as the pharmacological conditioning may contribute to an increase in coronary blood flow. Loke and Woodman[25] demonstrated that although IP was able to improve myocardial injury, it was not able to preserve vasodilator function. They also concluded that a reduction like this in the vasodilator reserve could not prevent adequate myocardial perfusion under conditions of elevated oxygen demands. In addition, under moderate

hypothermia, ischemic insult may induce a marked decrease in coronary flow that is associated with impaired myocardial protection.[26] However, Takeshima et al.[27] demonstrated that moderate hypothermia did not inhibit the preconditioning response.

Conclusion

We undertook this study because although hypothermia is widely used to protect the myocardium, IP is not the preferred treatment of choice in open heart surgery because in spite of offering significant improvement in the protection of myocardium, the postoperative low cardiac output is still a major concern, especially in high-risk patients. Therefore, the pharmacological induction of preconditioning, in contrast to classic IP, would be desirable, particularly in high-risk patients for whom an ischemic-type of preconditioning might further injure the diseased myocardium.

Our findings indicated that a short period of ischemic coronary blood flow during hypothermia and IP might lead to additive decreases in this flow. However, this might not be sufficient to prevent ischemic damage, and it could even be hazardous. Pretreatment with levosimendan provided better protection than IP in our study, especially under moderate hypothermic (28 °C) conditions. In addition, levosimendan and hypothermia provided additive protective effects in the ischemic myocardium.

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Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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REFERENCES

1. Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD. Bioenergetic consequences of opening the ATP-sensitive K(+) channel of heart mitochondria. Am J Physiol Heart Circ Physiol 2001;280:H649-57.
2. Daut J, Maier-Rudolph W, von Beckerath N, Mehrke G, Günther K, Goedel-Meinen L. Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. Science 1990;247:1341-4.
3. Grover GJ, Dzwonczyk S, Parham CS, Sleph PG. The protective effects of cromakalim and pinacidil on reperfusion function and infarct size in isolated perfused rat hearts and anesthetized dogs. Cardiovasc Drugs Ther 1990;4:465-74.
4. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124-36.
5. Kersten JR, Montgomery MW, Pagel PS, Warltier DC. Levosimendan, a new positive inotropic drug, decreases myocardial infarct size via activation of K(ATP) channels. Anesth Analg 2000;90:5-11.
6. Maytin M, Colucci WS. Cardioprotection: a new paradigm in the management of acute heart failure syndromes. Am J Cardiol 2005;96:26G-31G.
7. Pataricza J, Krassói I, Höhn J, Kun A, Papp JG. Functional role of potassium channels in the vasodilating mechanism of levosimendan in porcine isolated coronary artery. Cardiovasc Drugs Ther 2003;17:115-21.
8. Onem G, Sacar M, Aybek H, Kocamaz E, Adali F, Saçkan GK, et al. Protective effects of cilostazol and levosimendan on lung injury induced by lower limb ischemia-reperfusion. Turk Gogus Kalp Dama 2012;20:577-83.
9. Tritapepe L, De Santis V, Vitale D, Santulli M, Morelli A, Nofroni I, et al. Preconditioning effects of levosimendan in coronary artery bypass grafting--a pilot study. Br J Anaesth 2006;96:694-700.
10. Eisen A, Fisman EZ, Rubenfire M, Freimark D, McKechnie R, Tenenbaum A, et al. Ischemic preconditioning: nearly two decades of research. A comprehensive review. Atherosclerosis 2004;172:201-10.
11. Yapici D, Altunkan Z, Ozeren M, Bilgin E, Balli E, Tamer L, et al. Effects of levosimendan on myocardial ischaemia-reperfusion injury. Eur J Anaesthesiol 2008;25:8-14.
12. Yagi K. Lipid peroxides and related radicals in clinical medicine. Adv Exp Med Biol 1994;366:1-15.
13. Reading HW, Isbir T. Action of lithium on ATPases in the rat iris and visual cortex. Biochem Pharmacol 1979;28:3471-4.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
15. Weisel RD, Mickle DA, Finkle CD, Tumiati LC, Madonik MM, Ivanov J. Delayed myocardial metabolic recovery after blood cardioplegia. Ann Thorac Surg 1989;48:503-7.
16. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124-36.
17. Augoustides JG. The year in cardiothoracic and vascular anesthesia: selected highlights from 2008. J Cardiothorac Vasc Anesth 2009;23:1-7.
18. Bøtker HE, Kharbanda R, Schmidt MR, Bøttcher M, Kaltoft AK, Terkelsen CJ, et al. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. Lancet 2010;375:727-34.
19. Walsh SR, Tang TY, Kullar P, Jenkins DP, Dutka DP, Gaunt ME. Ischaemic preconditioning during cardiac surgery: systematic review and meta-analysis of perioperative outcomes in randomised clinical trials. Eur J Cardiothorac Surg 2008;34:985-94.

20. Ozyurt H, Irmak MK, Akyol O, Söğüt S. Caffeic acid phenethyl ester changes the indices of oxidative stress in serum of rats with renal ischaemia-reperfusion injury. Cell Biochem Funct 2001;19:259-63.
21. Meier P, Finch A, Evan G. Apoptosis in development. Nature 2000;407:796-801.
22. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493-501.
23. Maytin M, Colucci WS. Cardioprotection: a new paradigm in the management of acute heart failure syndromes. Am J Cardiol 2005;96:26G-31G.
24. Ozturk T, Gok S, Nese N. Levosimendan attenuates reperfusion injury in an isolated perfused rat heart model. J Cardiothorac Vasc Anesth 2010;24:624-8.
25. Loke KE, Woodman OL. Preconditioning improves myocardial function and reflow, but not vasodilator reactivity, after ischaemia and reperfusion in anaesthetized dogs. Clin Exp Pharmacol Physiol 1998;25:552-8.
26. Rohilla A, Kushnoor N, Kushnoor A. Myocardial ischemia reperfusion injury-pathogenesis and prevention. International journal of research in pharmacetuical and Biomedical sciences 2012;3:929-34.
27. Takeshima S, Vaage J, Löwbeer C, Valen G. Does hypothermia or hyperkalemia influence the preconditioning response? Scand Cardiovasc J 1999;33:79-87.