

Assessment of the Effects of Levosimendan and Nigella Sativa on Myocardial Ischemia Reperfusion Injury in Rats

Ratlarda Levosimendan ve Nigella Sativa'nın Miyokardiyal İskemi Reperfüzyon Hasarına Etkilerinin Değerlendirilmesi

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ABSTRACT

Objective: Ischemia-reperfusion injury is a chain of events put in place by tissue ischemia. Reperfusion following the damage of cell causes an active inflammatory response. In our research we tried to evaluate the protective effect of Levosimendan and Nigella Sativa on myocardial ischemia-reperfusion injury in rats.

Methods: We included twenty-four Wistar albino rats in our research. The rats were randomly divided into four experimental groups. The coronary arteries of rats in Group C (control group) were not occluded or reperfused. Left anterior descending coronary artery was ligated for 30 min to perform myocardial IR and then reperfused for 2 h in the IR (IR), IR-Levosimendan (24µg/kg) (IRL) and IR-Nigella Sativa (0.2 mL/kg) (IRNS) group.

Results: Inflammation findings were significantly higher in the IR group compared with the C, IR-NS, and IR-L groups ($p=0.001$, $p=0.019$, $p=0.019$, respectively). Compared with the C, IR-NS, and IR-L groups, the microscopic myocardial disorganization was significantly higher among the IR group ($p<0.0001$, $p=0.007$, $p=0.001$, respectively). The light microscopic myocardial tissue interstitial fibrosis levels were significantly higher in the IR group than in the C, IR-NS, and IR-L groups ($p<0.0001$, $p=0.044$, $p=0.003$, respectively).

Conclusion: Levosimendan and NS administration at the beginning of myocardial ischemia can provide varying degrees of protection against negative effects of variations in light microscopic inflammation findings, myocardial disorganization degrees and myocardial tissue interstitial fibrosis levels.

Key Words: Ischemia reperfusion, Levosimendan, Nigella Sativa, heart

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ÖZET

Giriş: İskemi-reperfüzyon hasarı, doku iskemisi tarafınca meydana gelen bir olaylar zinciridir. Hücre hasarını takiben reperfüzyon, aktif bir inflamatuvar yanıtı neden olur. Araştırmamızda, ratlarda Levosimendan ve Nigella Sativa'nın miyokardiyal iskemii-reperfüzyon hasarı üzerindeki koruyucu etkisini değerlendirmeyi amaçladık.

Yöntem: Araştırmamıza yirmi dört adet Wistar albino rat dahil ettik. Ratlar rastgele dört deney grubuna ayrıldı. Grup K'daki (kontrol grubu) ratların koroner arterleri oklude veya reperfüze edilmedi. Sol ön inen koroner arter miyokardiyal İR yapmak için 30 dakika bağlandı ve daha sonra İR (İR), İR-Levosimendan (24µg / kg) (İRL) ve İR-Nigella Sativa (0.2 mL / kg) (İRNS) gruplarında 2 saat reperfüze edildi.

Bulgular: İnflamasyon bulguları İR grubunda C, İR-NS ve İR-L gruplarına göre anlamlı olarak yüksek bulundu ($p=0.001$, $p=0.019$, $p=0.019$, sırasıyla). K, İR-NS ve İR-L grupları ile karşılaştırıldığında, mikroskopik miyokardiyal disorganizasyon, İR grubunda anlamlı olarak daha yüksekti ($p<0.0001$, $p=0.007$, $p=0.001$, sırasıyla). Işık mikroskopunda miyokardiyal doku interstisyel fibrozis düzeyleri İR grubunda K, İR-NS ve İR-L gruplarından anlamlı olarak daha yüksekti ($p<0.0001$, $p=0.044$, $p=0.003$, sırasıyla).

Sonuç: Miyokardiyal iskemii'nin başlangıcında Levosimendan ve NS uygulaması, ışık mikroskopunda inflamasyon bulguları, miyokardiyal düzensizlik dereceleri ve miyokardiyal doku interstisyel fibrozis düzeylerindeki değişimlerin olumsuz etkilerine karşı değişken derecelerde koruma sağlayabilir.

Anahtar Sözcükler: İskemi reperfüzyon, levosimendan, Nigella Sativa, kalp

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INTRODUCTION

An unavoidable systemic inflammatory response occurs after cardiopulmonary bypass mediated surgeries. Multiple organs are affected negatively caused by this systemic inflammatory response (1,2). The term, ischemia, to define remarkable decrease of rush of blood, oxygen and nutrients to the tissues. Reperfusion is important for tissue viability but reperfusion of ischemic tissues was proved to cause ischemia reperfusion injury (IRI) in a common way (3). Oxidative harm caused by IRI has a significant effect (4).

IRI gains an increased attention as the morbidity and mortality related to ischemic heart disease continues to increase. Therefore, fully figure out the science of IRI and seeking for novel therapeutic strategies is still the focus of intense research (5).

Levosimendan is a relatively new inotropic and vasodilator agent for the management of acute and chronic heart failure (6). It has vasodilator, positive inotropic and anti-ischemic effects (6-9). Levosimendan not only protects cardiac tissue against IRI but also reduces the risk of the spinal cord, lung and renal tissue damage (10-12).

Thymoquinone (TQ) is Nigella sativa (NS)'s main active ingredient. It is generally called as black cummin or black seed. Black seed is an annual flowering plant native to some areas like Mediterranean countries (13). 1963 was the year thymoquinone was first extracted as the main active ingredient of NS (14) and it was described as a potent superoxide scavenger and free radical (15-17). However, NS also has an antioxidative effect on the spinal cord, heart and renal tissue IRI (18-20).

The effects of levosimendan and NS on myocardial IRI have not yet been investigated to the best of our knowledge. We aimed to investigate the protective effect of levosimendan and NS in an experimental rat model of myocardial IRI.

MATERIALS and METHODS

Animals and Experimental Protocol

After the approval of the Experimental Animals Ethics Committee of Gazi University the study was carried out in the GUDAM Laboratory of Gazi University. All employed methods were in agreement with approved basics of the Guide for the Care and Use of Laboratory Animals. Their weight varied between 250 and 300 g. At least one week before the surgery in a pathogen free environment we housed the animals in standard cages. During this time they were free to access food (until 2 h earlier than the procedure of anesthesia) and water. Under 12 h dark-light cycle and the animals were separated into four groups of six rats randomly. IP 100 mg.kg⁻¹ ketamine was used for the anesthesia of rats. For artificial respiration cannulation of trachea was done. Each of the rats was fixed in a supine position after their chest was shaved. Fourth and fifth ribs were sectioned nearly 2 mm to the left of the sternum after left thoracotomy. 1.5 ml volume /100 g body weight with room air was used for positive pressure artificial respiration at a rate of 60 strokes/min. Through the peripheral vein in the tail sodium heparin (500 IU/kg) was given.

With a soft pressure to the right rib cage after incising pericardium heart was taken out. Attached to 10-mm micropoint reverse-cutting needle a 8/0 silk suture was rapidly put under the left main coronary artery. 20 min was waited after the heart was put back in chest for the recovery of the rat.

Whenever intermittent tail pinch or positive reaction to surgical stress was detected during the experiment 20 mg.kg⁻¹ recurrent injections of ketamine was used to maintain anesthesia.

There were four groups in the research: Group C (control; n=6), Group IR (IR; n=6), Group IRNS (IR-nigella sativa; n=6) and Group IRL (IR-levosimendan; n=6). In the IRL group left thoracotomy was done and levosimendan was administered (Simdax 2.5 µg/ml, Abbott®, Orion Pharma, Espoo, Finland) 24 µg.kg⁻¹ diluted in 10 ml of 0.5% dextrose intraperitoneally 30 min prior to ligation of the LAD. Left thoracotomy was done in the IRNS group and IP 0.2 mL.kg⁻¹ nigella sativa (Tymoquinone 1G, Sigma Aldrich®) was administered by intraperitoneal route 30 min prior to ligation of the LAD. A small plastic snare was placed through the ligature and put onto the heart. By applying tension to the ligature the artery was occluded for 30 min, and then tension was released. Reperfusion was lasted for 120 min. However, after the procedure mentioned above, no occlusion or reperfusion of the coronary artery was performed in the control rats. Histopathological evaluation of heart tissue specimen was done at the end of reperfusion period. At the end of the experiment rats were decapitated.

Histological determinations

10% buffered neutral formalin was used to fix the specimens. Then the specimens were embedded in paraffin. From apex to the base heart was separated into four segments to visualize lesions of myocardium at different levels. 4-µm thickness cross-sections were taken from each of the segments after the divided segments were embedded in paraffin.

Hematoxylin-Eosin (Bio-optica, Milano, Italy) was used to stain the slides to evaluate the histological features of tissues and then examined under light microscope for myocardial disorganization, inflammation, and interstitial fibrosis. At least examination of 10 fields for each slide were done. Severity of changes was graded using scores on a scale of (-), none; (+), mild; (++) moderate; (+++), severe.

Statistical Analysis

Statistical analyses were performed with SPSS (Chicago, IL, USA) 20.0 program. P-value <0.05 was considered statistically significant. To determine which group differs from the others and if the results of the ANOVA test were significant, we used the Bonferroni-adjusted test. The data were expressed as mean ± standard deviation (mean ± SD).

RESULTS

Findings of light microscopic inflammation were significantly different among groups (p=0.006). In the DIR group compared to the C findings related to inflammation were remarkably higher in the DIR group, IR-NS, and IR-L groups (p=0.001, p=0.019, and p=0.019, respectively) (Table 1, Figs. 1-4). In a similar way, remarkable difference of myocardial disorganization degrees was seen in light microscope among groups (p<0.0001). Compared to the C, IR-NS, and IR-L groups, the microscopic myocardial disorganization was remarkably higher in the IR group (p<0.0001, p=0.007, and p=0.001, respectively) (Table 1, Figs. 1-4). Interstitial fibrosis of the myocardial tissue was remarkably different among groups (p=0.001). Levels of interstitial fibrosis of myocardial tissue in the light microscope were remarkably higher in the IR group than in the C, IR-NS, and IR-L groups (p<0.0001, p=0.044, and p=0.003, respectively) (Table 1, Figs. 1-4).

Table1: Histopathological findings of the heart tissue (Mean ± SE)

	Group C (n=6)	Group IR (n=6)	Group IR-NS (n=6)	Group IR-L (n=6)	P**
Inflammation	0.17±0.17*	2.00±0.37	0.83±0.31*	0.83±0.40*	0.006
Myocardial disorganization	0.33±0.21*	2.00±0.26	1.00±0.26*	0.67±0.21*	<0.0001
Interstitial fibrosis	0.17±0.17*	2.00±0.26	1.17±0.31	0.67±0.33*	0.001

p**: A p value < 0.05 was statistically significant for Kruskal-Wallis test

* p<0.05: Compared to group IR

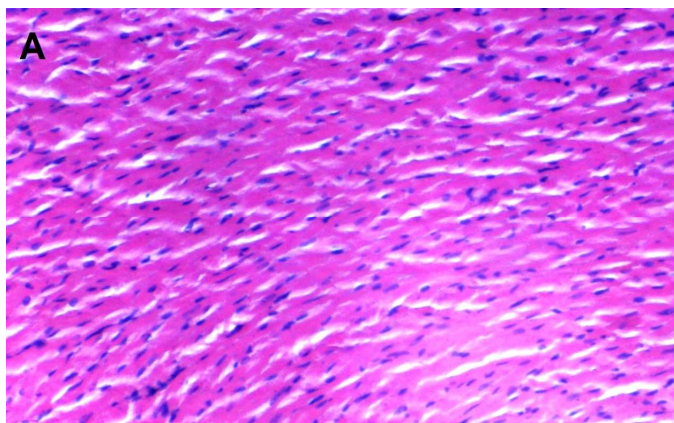


Figure 1: Normal-structured myocardial tissue fibers in the control group, HEx100.

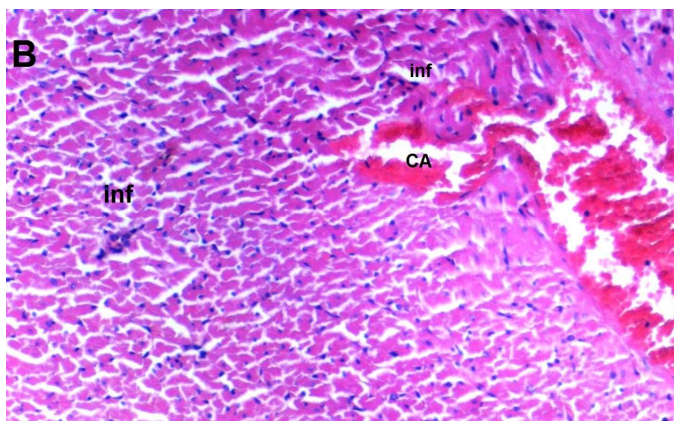


Figure 2A: Extensive inflammatory cell infiltration and edema myocardial tissue. Inflammatory cell infiltration and fibrosis among cardiac muscle cells CA- peri-coronary arter fibrosis. Increased connective tissue among myocardial fibres in the ischemia reperfusion group, HEx100.

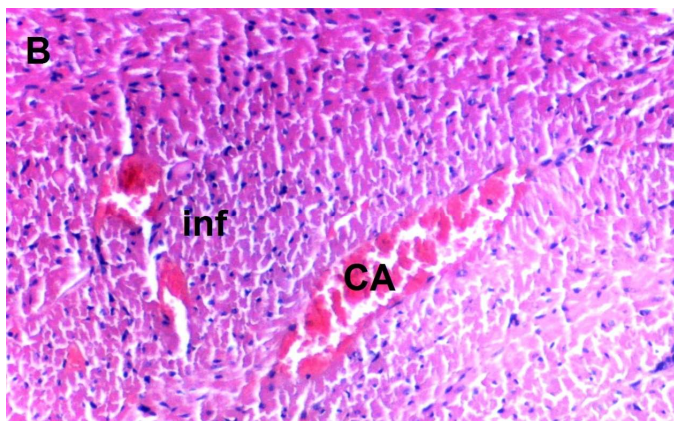


Figure 2B: Extensive inflammatory cell infiltration and edema myocardial tissue. Inflammatory cell infiltration and fibrosis among cardiac muscle cells CA- peri-coronary arter fibrosis. Increased connective tissue among myocardial fibres in the ischemia reperfusion group, HEx100.

DISCUSSION

Levosimendan as a calcium sensitizer causes increased contractility of myocardium (21). Thus, it increases cardiac output and ejection fraction. Also because of its positive inotropic, lusitropic and vasodilatory properties levosimendan has a reducing effect on cardiac filling pressures, systemic, pulmonary and coronary vascular resistance (7,22,23). In addition, levosimendan has a relaxing effect on vascular smooth muscle by opening adenosine triphosphate-sensitive potassium channels (23,24).

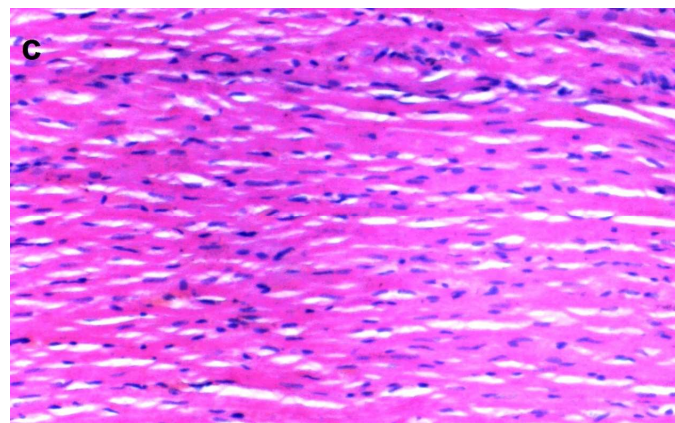


Figure 3: Sparse connective tissue among myocardial fibres and mild myocardial disorganization are noticed in the ischemia reperfusion nigella sativa group, HEx100.

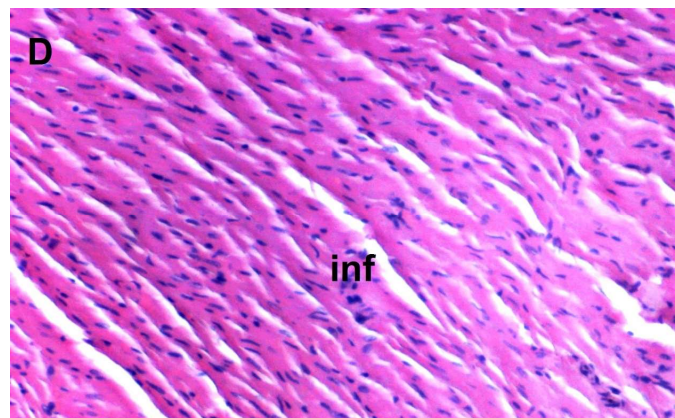


Figure 4 Sparse connective tissue among myocardial fibres and mild myocardial disorganization are noticed in the ischemia reperfusion levosimendan group, HEx100.

Hosseinzadeh et al showed that TQ has protective effects on lipid peroxidation after IRI in rat hippocampus (25). In various experimental studies, TQ's protective effects including antioxidant and free radical scavenging activity were shown (15-17). Additionally, TQ inhibits the some inflammatory mediators' production (26-32). Following IRI, NS seed oil treatment was shown to improve renal function, serum and tissue anti-oxidative parameters and reduce renal histopathological score by Bayrak et al (33).

Myocardial ischemia is one the most challenging diseases as there is a large clinical variability in terms of severity of ischemia. The pathogenesis of the IRI-induced myocardial damage is caused by both ischemia and reperfusion and appears to be multifactorial. It includes hypoxia, excessive production of reactive oxygen species and inflammatory response.

In our study, we have determined that levosimendan and NS administration at the beginning of myocardial ischaemia can provide varying degrees of protection against negative effects of variations in light microscopic inflammation findings, myocardial disorganization degrees and myocardial tissue interstitial fibrosis levels.

To conclude, this study's results obviously indicated that administration of levosimendan and NS before the induction of ischemia had protective effects on these alterations in myocardial IRI. We still think these promising results should be further supported by more detailed studies with larger volumes.

Conflict of interest

No conflict of interest was declared by the authors.

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