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

Abdullah Özer¹, Yiğit Kılıç¹, Şaban Cem Sezen², Ayşegül Küçük³, Barış Mardin¹, Metin Alkan⁴, Mustafa Arslan⁴, Yusuf Ünal⁴, Levent Oktar¹

¹Department of Cardiovascular Surgery, Gazi University Medical Faculty, Ankara, Turkey
²Department of Histology and Embryology, Kirikkale University Medical Faculty, Kirikkale, Turkey
³Department of Physiology, Dumlupınar University Medical Faculty, Kutahya, Turkey
⁴Department of Anaesthesiology and Reanimation, Gazi University Medical Faculty, Ankara, Turkey

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

Received: 04.04.2018 Accepted:05.21.2018

ÖZET

Giriş: İskemi-reperfüzyon hasarı, doku iskemisi tarafınca meydana gelen bir olaydır zinicidir. Hücre hasarını takiben reperfüzyon, aktif bir inflamatuar yanıt neden olur. Araştırmamızda, ratlarda Levosimendan ve Nigella Sativa’nın miyokardiyal iskemi-reperfüzyon hasarı üzerindeki etkisini değerlendirdik.

Yöntem: Araştırmamıza yirmi dört adet Wistar albino rat dahil ettik. Ratlar rastgele dört deney grubuna ayrılır. Sol ön inen koroner arter bağlanır, miyokardiyal IR gerçeklenir ve daha sonra IR (İR), IR-Levosimendan (24ug / kg) (İRL) ve IR-Nigella Sativa (0.2 mL / kg) (IRNS) gruplarında 2 saat reperfüze edildi.

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

Sonuç: Miyokardiyal iskeminin başlangıcında Levosimendan ve NS uygulaması, işık mikroskopunda inflamasyon bulguları, miyokardiyal disorganizasyon dereceleri ve miyokardiyal doku interstisyal fibrozis düzeylerindeki değişiminin olumsuz etkilerine karşı değişik derecelerde koruma sağlayabilir.

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

Geliş Tarihi:04.04.2018 Kabul Tarihi:21.05.2018
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 cumin 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 levsosimendan and NS on myocardial IRI have not yet been investigated to the best of our knowledge. We aimed to investigate the protective effect of levsosimendan 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. For artificial respiration cannulation of trachea 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. 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 to maintain anesthesia. From 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, remarkably higher 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). Intersitial 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).

Table 1: 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
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 vasodilatatory 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).
5. Guo J, Wang SB, Yuan TY, Wu YJ, Yan Y, Li L, e al. Coptisine protects rat heart against myocardial ischemia/reperfusion injury by suppressing myocardial apoptosis and inflammation. Atherosclerosis 2013;231:384-91.

6. Rognoni A, Lupi A, Lazzero M, Bongo AS, Rognoni G. Levosimendan: from basic science to clinical trials. Recent Pat Cardiovasc Drug Discov 2011;6:9-15.

7. Toller WG, Stranz C. Levosimendan, a new inotropic and vasodilatory agent. Anesthology 2006;104:556-69.

8. Erdei N, Papp Z, Pollesello P, Edes I, Bagi Z. The levosimendan metabolite OR-1986 elicits vasodilation by activating the K(ATP) and BK(Ca) channels in rat isolated arterioles. Br J Pharmacol 2006;148:696-702.

9. Keheinen P, Pollesello P, Leviyki J, Haikala H. Levosimendan increases diastolic coronary flow in isolated guinea pig heart by opening ATP-sensitive potassium channels. J Cardiovasc Pharmacol 2001;37:367-74.

10. Katircioglu SF, Seren M, Parlar AI, Turan MN, Manavbasi Y, Aydog G, et al. Levosimendan effect on spinal cord ischemia-reperfusion injury following aortic clamping. J Card Surg 2008;23:44-8.

11. Yasa H, Yakut N, Emrecan B, Ergunes K, Ortac R, Karahan N, et al. Protective effects of levosimendan and iloprost on lung injury induced by limb ischemia-reperfusion: A rabbit model. J Surg Res 2008;147:138-42.

12. Yakut N, Yasa H, Bahriye Lafci B, Ortac R, Tulukoglu E, Aksun M, et al. The influence of levosimendan and iloprost on renal ischemia-reperfusion: An experimental study. Interact Cardiovasc Thorac Surg 2008;7:235-9.

13. Gali-Muhtasib H, Roessner A, Schneider-Stock R. Thymoquinone: a promising anti-cancer drug from natural sources. Int J Biochem Cell Biol 2006;38:1249–53.

14. El-Dakhakhny M. Studies on the chemical constitution of Egyptian N. sativa L. seeds. Planta Med 1963;11:465–70.

15. Mansour MA, Nagi MN, El-Khatib AS, Al-Bekairi AM. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. Cell Biochem Funct 2002;20:143–51.

16. Gökce EC, Kahveci R, Gökce A, Cemil B, Aksoy N, Sargon MF, et al. Neuroprotective effects of thymoquinone against spinal cord ischemia-reperfusion injury by attenuation of inflammation, oxidative stress, and apoptosis. J Neurosurg Spine 2016;24:949-59.

17. Gonca E, Kurt Ç. Cardioprotective effect of Thymoquinone: A constituent of Nigella sativa L., against myocardial ischemia/reperfusion injury and ventricular arrhythmias in anaesthetized rats. Pak J Pharm Sci 2015;28:1267-73.

18. Hammad FT, Lubbad L. The effect of thymoquinone on the renal functions following ischemia-reperfusion injury in the rat. Int J Physiol Pathophysiol Pharmacol 2016;25;8:152-9.

19. Papp Z, Edes I, Fruhwald S, De Hert SG, Salmenperä M, Leppikangas H, et al. Levosimendan: molecular mechanisms and clinical implications. Consensus of experts on the mechanisms of action of levosimendan. Int J Cardiol 2012;159:82-7.

20. Lehtonen L, Pöder P. The utility of levosimendan in the treatment of heart failure. Ann Med 2007;39:2-17.

21. Papp Z, Csapó K, Pollesello P, Haikala H, Edes I. Pharmacological mechanisms contributing to the clinical efficacy of levosimendan. Cardiovasc Drug Rev 2005;23:71-98.

22. Katrancioglu N, Karahan O, Kilic AL, Altun A, Katrancioglu O, Polat ZA. The antiangiogenic effects of levosimendan in a CAM assay. Microvasc Res 2012;83:263-6.

23. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Effect of thymoquinone and Nigella sativa seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine 2007;14:621-7.

24. Banerjee S, Kaseb AO, Wang Z, Kong D, Mohammad M, Padhye S, et al. Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. Cancer Res 2009;69:5575–83.

25. Chehl N, Chiptsyina G, Gong Q, Yeo CI, Arafat HA. Anti-inflammatory effects of the Nigella sativa seed extract, thymoquinone, in pancreatic cancer cells. Cancer Res 2009;69:1137–81.

26. El Gazzar MA, El Mezayen R, Nicolls MR, Dreskin SC. Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. Biochim Biophys Acta 2007;1770:556–64.

27. Eshelby NM, El-Sherbiny M. Thymoquinone attenuates Doxorubicin-induced nephrotoxicity in rats: role of NrF2 and NOX4. Chem Biol Interact 2008;20:122-102.

28. El Gazzar MA, El Mezayen R, Nicolls MR, Dreskin SC. Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. Biochim Biophys Acta 2007;1770:556–64.

29. Elsherbiny NM, El-Sherbiny M. Thymoquinone attenuates Oxalobin-induced nephrotoxicity in rats: role of NrF2 and NOX4. Chem Biol Interact 2012;204:122-102.

30. Li F, Rajendran P, Sethi G. Thymoquinone inhibits proliferation, induces apoptosis and chemosensitizes human multiple myeloma cells through suppression of signal transducer and activator of transcription 3 activation pathway. Br J Pharmacol 2010;161:541–54.

31. Rajput S, Kumar BN, Banik P, Parida S, Mandal M. Thymoquinone restores radiation-induced TGF-beta expression and abrogates EMT in chemoradiotherapy of breast cancer cells. J Cell Physiol 2015;230:620–9.

32. Samarghandian S, Azimi-Nezhad M, Mehrad-Majd H, Mirhafiz SR. Thymoquinone ameliorates acute renal failure in gentamicin-treated adult male rats. Pharmacology 2015;96:112–7.

33. Bayrak O, Babvek N, Karatas OF, Bayrak R, Catal F, Cimentepe E, et al. Nigella sativa protects against ischemia/reperfusion injury in rat kidneys. Nephrol Dial Transplant. 2008;23:2206–12.