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The effect of morphine delta receptor activity on ischemic postconditioning in lung ischemia reperfusion injury

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Abstract:

OBJECTIVE: In the context of the physiopathology of lung damage due to ischemia and reperfusion injury, we aimed to reveal the effects of the addition of morphine sulfate to ischemic postconditioning (PC) protocol.

METHODS: In the present study, 48 Wistar albino female rats were employed. Group 1 was accepted as the Sham group that underwent thoracotomy through the fifth left intercostal space. Ischemia-reperfusion (IR) group: Thoracotomy and IR period. IRPC group: thoracotomy, IR period and ischemic PC. In IRPC3 and IRPC30 groups, in addition to ischemic PC different doses of morphine sulfate (3 μmol and 30 μmol) was administered. Tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-10 levels were measured in the biochemical assessment of the lung tissue samples obtained.

RESULTS: TNF-α and IL-1 (pro-inflammatory cytokines) have lower values, and IL-10 (anti-inflammatory cytokine) have higher values both in the groups which have been subject to PC and morphine. TNF-α and IL-1 levels in lung tissue were statistically significant between the IRPC3 group and the IR and IRPC groups. In addition, IL-10 level in lung tissue was statistically significant between the IRPC3 group and the IRPC group.

CONCLUSION: In the present study conducted with experimental animals where morphine was also injected beside ischemic PC protocols, statistically significant differences were determined in the lung tissue analyses when we compared pro-inflammatory and anti-inflammatory cytokine values. We firmly believe that adding morphine to the lung transplantation protocols and PC will decrease IR damage.

Keywords:

Ischemia and reperfusion injury, ischemic postconditioning, morphine sulfate

Introduction

Ischemia-reperfusion (IR) injury is a complex pathological process that begins with the absence of oxygen in the tissue, continues with the production of free-oxygen radicals and expands as an inflammatory response.[1] Instead of long reperfusion after ischemia, reperfusion at short-term intervals could be more effective in decreasing total IR (IR) injury. This phenomenon is called ischemic post-conditioning (PC).

Opioids have beneficial and undesirable effects on almost every organ and function in the human body. The most important targets of opioids are the central nervous system and gastrointestinal system. However, cardiovascular, pulmonary, genitourinary, and immunological systems are also directly affected. In studies performed, opioid delta receptor activation was found to significantly increase the lifespan in rats subjected to body hypoxia.[2] In the literature, there are no studies investigating the effect of morphine sulfate on pulmonary IR injury. In our study, we aimed to reveal

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the effects of the addition of morphine sulfate to ischemic PC protocol.

Methods

The study was approved by the Local Experimental Medical Research and Application Centre-Experimental Animals Ethics Committee. A total of 48 Wistar Albino female rats were obtained from the Experimental Animal Laboratory of Local Experimental Medical Research and Application Centre.

Study design

The subjects were divided into a total of five groups with one group of 8 called the Sham group, and other four groups of 10 rats.

- Sham group (n = 8): Left thoracotomy
- IR group (n = 10): Left thoracotomy and IR
- IRPC group (n = 10): Left thoracotomy, IR period and PC
- IRPC3 group (n = 10): Left thoracotomy, IR period and PC + morphine sulfate (3 μmol)
- IRPC30 group (n = 10): Left thoracotomy, IR period and PC + morphine sulfate (30 μmol).

Thoracotomy was performed from the fifth left intercostal space in the sham group, but IR period and PC were not performed. In the IR group, thoracotomy was performed from the fifth intercostal space on the left, and IR period was established, but PC was not performed. In the IRPC group, IR period and PC were performed as well as thoracotomy from the fifth left intercostal space. Morphine sulfate at doses of 3 μmol and 30 μmol, respectively, were administered to the experimental animals of the IRPC3 and IRPC30 groups over 20 min starting 5 min before reperfusion by using the morphine sulfate venous route. Experimental animals were sacrificed by administering an anesthetic at a high dose (150 mg/kg ketamine/30 mg/kg xylazine) intraperitoneally at the 128th min.[3] The left lung was removed from the thoracic cavity, and the surface was cleaned with physiological saline. Lung tissue was stored at −80°C in buffered phosphate solution for biochemical assays.[4,5]

Experimental protocol

A total of 50 mg/kg ketamine (Ketalar vial., Pfizer Pharma GMBH, Germany) and 10 mg/kg xylazine hydrochloride (Alfazyne 2%, Alfasan International, Holland) were administered as anesthetics intraperitoneally. When necessary, ketamine (half dose, 25 mg/kg) was repeated with reflex responses (pedal reflex, palpebral, and corneal reflexes) to keep the anesthesia depth constant. Body temperature was monitored by inserting a heat probe into the ECG and rectum by the aid of needle electrodes. Heating lamps were used to keep the animals at 37°C ± 5°C body temperature during the surgical preparation and working period.

After passing the skin and the subcutaneous layer through the midline of the neck, the muscle layers were dissected, and the internal jugular vein was catheterized with 24G cannula for fluid resuscitation. Detection was made by ligating the vein wall with a silk suture that was used for hanging. Braun 8871 Compact Perfuser and Braun Perfuser injector were used for fluid resuscitation from the left jugular vein with 4 ml/kg/saline in all rats throughout the experiment. The surrounding tissue was then dissected up to the trachea. The tracheostomy was opened and implanted with a 16G plastic catheter and connected to the respiratory tract. Each rat was placed in a fixed position on a smooth surface and prepared for surgery. During the operation, the rats were given ventilatory support in the volume control mode. The ventilation rate was set at 90/min, and the volume was adjusted at 10 ml/kg/min. We planned the average duration of anesthesia to be 128 min for each rat.

The left thoracotomy was performed from the 5th intercostal space, and the ribs were excised posteriorly. This provided a wide field of vision for the heart and lungs. The thorax was explored and released by cutting the inferior ligament. The left lung was retracted to the lower larynx and left hilar structures were removed. Left pulmonary artery, vein and bronchus were divided. The lungs were clamped in a semi ventricular position from near the heart by hilar nontraumatic microvascular clamps (Vascu-Statt REF 1001-535).

Postconditioning protocol

All rats were given 500 U/kg of intravenous heparin in the 1st min. After 5 min of heparin administration, the left hilus was occluded using a nontraumatic vascular bulldog clamp (Vascu-Statt REF 1001-535) at 6 min, and ischemia was induced for 60 min. The lungs were kept moist with periodic applications of the warm sterile saline solution, and the incision was closed with a gauze pad impregnated with sterile hot saline solution to minimize evaporation loss. At the 1st min of reperfusion, PC algorithm was initiated; ischemia was created with a vascular clamp for 30 s, followed by reperfusion for 30 s, followed by ischemia for 30 s and reperfusion for 30 s for the second time. After the third and last 30 s of ischemia, reperfusion was performed for 60 min, and the experiment was then terminated by high-dose anesthetic administration at the 128th min after removing the lung tissue. Three rats which were dead before the end of the experiment were withdrawn from the experiment.

Biochemical measurements

After weighing the frozen lung samples of the rats, they were homogenized in an ice bath for 1 min with a homogenizer (Ultra-Turrax T25, Germany) in a 100 mmol/L phosphate buffer containing 0.05% sodium azide in 4.5 ml of pH 7.4. The 10% homogenates obtained

at the end of the process were sonicated in an ice bath for 30 s (Bandelin Sonoplus UW 2070, Germany). The samples were then centrifuged at 4000 g for 10 min in a refrigerated centrifuge (Eppendorf MR 5415, Germany) at +4°C to obtain supernatants. Homogenization work was done at Necmettin Erbakan University Medical Biochemistry Department. Tumor necrosis factor (TNF)-α, IL-1, and IL-10 levels in the biochemical evaluation were expressed in pg/mg (picogram/milligram) in the lung tissue.

Table 1: Biochemical parameters values of lung tissue

Lung tissue	Number of subjects	Group average	SD	Minimum value	Maximum value
TNF-α					
Sham	8	46.473	25.7378	27.8	92.6
IR	10	55.250	13.2102	28.8	63.5
IRPC	10	43.807	14.6427	20.6	65.8
IRPC3	10	22.535	9.166	11.3	38.5
IRPC30	10	25.491	8.5367	16.4	37.9
IL-1					
Sham	8	33.619	9.6229	21.2	59.6
IR	10	47.139	6.8545	23.7	73.6
IRPC	10	27.704	4.3979	20.1	34.8
IRPC3	10	19.583	3.4808	15.5	24.1
IRPC30	10	15.067	4.3783	8.10	26.2
IL-10					
Sham	8	35.784	11.3722	23.8	67.7
IR	10	26.324	8.3375	18.1	60.1
IRPC	10	35.248	11.97	19.2	67.5
IRPC3	10	47.284	7.1867	24.2	65.8
IRPC30	10	49.323	7.6824	25.9	78.5

IR: Ischemia reperfusion, IRPC: Ischemia reperfusion and ischemic postconditioning, IRPC3 and IRPC30: Ischemia reperfusion, ischemic postconditioning and morphine sulfate (3 μmol and 30 μmol), TNF-α: Tumor necrosis factor alpha, SD: Standard deviation, IL: Interleukin

Statistical evaluation

SPSS 15.0 program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The results were expressed as a mean ± standard deviation. Analysis of variance test was used to compare multiple groups in evaluating biochemical results. When the test result was significant, the Tukey honestly significant test was used to compare groups. Significance level was accepted as $P < 0.05$ in all statistical analyses.

Results

The highest values of TNF-α, IL-1, and IL-10 were determined in the sham group while the lowest values were observed in the IRPC3 or IRPC30 group [Table 1].

Tissue TNF-α values were found to differ from group to group. A statistically significant difference was

Table 2: Statistical evaluation of tumor necrosis factor alpha levels in lung tissue according to groups

Groups	Difference between groups	SE	*P*	95% CI	
				Minimum value	Maximum value
Sham					
IR	8.7771	7.2492	0.764	−12.550	30.104
IRPC	11.4435	7.4492	0.546	−9.884	32.771
IRPC3	32.7152*	7.6492	0.001*	11.388	54.043
IRPC30	29.7595*	7.6651	0.003*	7.814	51.705
IR					
Sham	−8.7771	7.4492	0.764	−30.104	12.550
IRPC	2.6664	7.2267	0.996	−18.024	23.357
IRPC3	23.9381*	7.2267	0.016*	3.248	44.629
IRPC30	20.9825	7.4492	0.046*	−0.345	42.310
IRPC					
Sham	−11.4435	7.1492	0.546	−32.771	9.884
IR	−2.6664	7.2267	0.996	−23.357	18.024
IRPC3	21.2717*	7.2267	0.041*	0.581	41.962
IRPC30	18.3161	7.4492	0.122	−3.011	39.643
IRPC3					
Sham	−32.7152*	7.4492	0.001*	−54.043	−11.388
IR	−23.9381*	7.2267	0.016*	−44.629	−3.248
IRPC	−21.2717*	7.2267	0.041*	−41.962	−0.581
IRPC30	−2.9557	7.4492	0.995	−24.283	18.372
IRPC30					
Sham	−29.7595*	7.6651	0.003*	−51.705	−7.814
IR	−20.9825	7.4392	0.056	−42.310	0.345
IRPC	−18.3161	7.4492	0.122	−39.643	3.011
IRPC3	2.9557	7.4192	0.995	−18.372	24.283

IR: Ischemia reperfusion, IRPC: Ischemia reperfusion and ischemic postconditioning, IRPC3 and IRPC30: Ischemia reperfusion, ischemic postconditioning and morphine sulfate (3 μmol and 30 μmol), CI: Confidence interval, SE: Standard error, TNF-α: Tumor necrosis factor alpha

found between the sham group and the IRPC3 and IRPC30 groups, between the IR group and the IRPC3 and IRPC30 groups, and between the IRPC group and the IRPC3 group. Tissue TNF-α values were different in the other two group comparisons, but no statistically significant difference was found [Table 2].

There was a statistically significant difference between the groups of Sham and IR, IRPC, IRPC3, IRPC30, between IR group and IRPC3 and IRPC30 groups, and between IRPC and IRPC3 and IRPC30 groups regarding tissue IL-1 levels. Tissue IL-1 levels were different in the other two group comparisons, but no statistically significant difference was found [Table 3].

Regarding tissue IL-10 levels, there was a statistically significant difference between the groups of Sham and IR, IRPC, IRPC3, IRPC30, between IRPC and IRPC3 and IRPC30 groups. Tissue IL-10 levels were different in the other two-group comparisons, but not statistically significant [Table 4].

Discussion

Ischemic preconditioning is a phenomenon that is defined as making resistance to harmful effects of prolonged ischemia and reperfusion by exposure to short periods of IR before the period of severe ischemia. This phenomenon was first shown in 1986 by Murry *et al*. in cardiac tissue.[6] Although there is evidence that preconditioning reduces IR damage in solid organs such as the heart, liver, kidneys, and bones, there are limited data about the lungs.[7] Ischemic preconditioning in the lungs reduces IR damage by decreasing neutrophil count, thromboxane B2 and malondialdehyde levels, increasing superoxide dismutase and calcitonin gene-dependent peptide levels, and decreasing apoptosis of lung cells.[8]

The concept of ischemic PC is defined as a series of short repetitive mechanical interruptions of reperfusion at the beginning of reperfusion which is then followed by a specified algorithm. The algorithm takes about 1–3 min depending on the type.[9] In this study, we started the PC algorithm in the 1st minute by creating 30 s of ischemia with a vascular clamp, reperfusion for the following 30 s, then creating ischemia for the second time for 30 s and reperfusion for another 30 s, and then we performed reperfusion for 60 min.

Staat *et al*. have shown that PC following coronary angioplasty and stenting maintains the peak infarct volume during acute myocardial infarction.[10] It has

Table 3: Statistical evaluation of ınterleukin-1 levels in lung tissue according to groups

Groups	Difference between groups	SE	*P*	95% CI	
				Minimum value	Maximum value
Sham					
IR	13.5202*	2.0398	0.000*	5.046	21.994
IRPC	19.4352*	2.9598	0.000*	10.961	27.909
IRPC3	27.5554*	2.3498	0.000*	19.081	36.030
IRPC30	32.0721*	3.0456	0.000*	23.352	40.792
IR					
Sham	−13.5202*	2.9698	0.000*	−21.994	−5.046
IRPC	5.9150	2.8714	0.258	−2.306	14.136
IRPC3	14.0352*	2.8714	0.000*	5.814	22.256
IRPC30	18.5519*	2.9598	0.000*	10.078	27.026
IRPC					
Sham	−19.4352*	2.9598	0,000*	−27.909	−10.961
IR	−5.9150	2.8714	0.258	−14.136	2.306
IRPC3	8.1202	2.8714	0.044*	−0.101	16.341
IRPC30	12.6369*	2.9098	0.001*	4.163	21.111
IRPC3					
Sham	−27.5554*	2.9598	0.000*	−36.030	−19.081
IR	−14.0352*	2.8714	0.000*	−22.256	−5.814
IRPC	−8.1202	2.8714	0.044*	−16.341	0.101
IRPC30	4.5167	2.9198	0.553	−3.957	12.991
IRPC30					
Sham	−32.0721*	3.0456	0.000*	−40.792	−23.352
IR	−18.5519*	2.9598	0.000*	−27.026	−10.078
IRPC	−12.6369*	2.9298	0.001*	−21.111	−4.163
IRPC3	−4.5167	2.9598	0.553	−12.991	3.957

IR: Ischemia reperfusion, IRPC: Ischemia reperfusion and ischemic postconditioning, IRPC3 and IRPC30: Ischemia reperfusion, ischemic postconditioning and morphine sulfate (3 μmol and 30 μmol), CI: Confidence interval, SE: Standard error, IL: Interleukin

Table 4: Statistical evaluation of ınterleukin-10 levels in lung tissue according to groups

Groups	Difference between groups	SE	*P*	% 95 CI	
				Minimum value	Maximum value
Sham					
IR	32.1671*	4.6192	0.000*	18.942	45.392
IRPC	30.1286*	4.6192	0.000*	16.904	43.354
IRPC3	44.2032*	4.6192	0.000*	30.978	57.428
IRPC30	43.6685*	4.7531	0.000*	30.060	57.277
IR					
Sham	−32.1671*	4.6192	0.000*	−45.392	−18.942
IRPC	−2.0385	4.4813	0.991	−14.869	10.792
IRPC3	12.0361	4.4813	0.075	−0.794	24.866
IRPC30	11.5014	4.6192	0.114	−1.724	24.726
IRPC					
Sham	−30.1286*	4.6192	0.000*	−43.354	−16.904
IR	2.0385	4.4813	0.991	−10.792	14.869
IRPC3	14.0746*	4.4813	0.025*	1.245	26.905
IRPC30	13.5399*	4.6192	0.043*	0.315	26.765
IRPC3					
Sham	−44.2032*	4.6192	0.000*	−57.428	−30.978
IR	−12.0361	4.4813	0.075	−24.866	0.794
IRPC	−14.0746*	4.4813	0.025*	−26.905	−1.245
IRPC30	−0.5347	4.6192	1.000	−13.760	12.690
IRPC30					
Sham	−43.6685*	4.7531	0.000*	−57.277	−30.060
IR	−11.5014	4.6142	0.114	−24.726	1.724
IRPC	−13.5399*	4.6192	0.043*	−26.765	−0.315
IRPC3	0.5347	4.6132	1.000	−12.690	13.760

IR: Ischemia reperfusion, IRPC: Ischemia reperfusion and ischemic postconditioning, IRPC3 and IRPC30: Ischemia reperfusion, ischemic postconditioning and morphine sulfate (3 μmol and 30 μmol), CI: Confidence interval, SE: Standard error, IL: Interleukin

been shown in several other recent studies that similar activity may also be in the brain.[11-13] It has been shown that PC is modified reperfusion and protects the cerebral tissue against IR injury.[12] Hence in many studies that have been done both in the heart and in the brain, it has been shown that PC reduces IR damage. In our study, we measured the levels of inflammatory cytokines such as TNF-α, IL-1, and IL-10 in lung tissue by generating ischemic PC during reperfusion. We have seen that TNF-α and IL-1 (pro-inflammatory cytokines) have lower values and IL-10 (anti-inflammatory cytokine) have higher values both in the groups which have been subject to PC and morphine.

TNF-α and IL-1 are pro-inflammatory cytokines that play a key role in IR mediated inflammatory processes. It is known that TNF-α and IL-1 act through similar co-signaling molecules that appear early in inflammation. TNF-α contributes to neutrophil-mediated endothelial damage by causing neutrophil activation and release of endothelial leukocyte adhesion molecules. Endothelial damage is caused by elastase and free oxygen radicals released from active neutrophils. Moreover, TNF-α and IL-1 trigger apoptosis and control the production of IL-6 secreted by phagocytes, T cells, and endothelial cells.[14]

Güneş *et al*. found that TNF-α and IL-1 levels were lower in rats subject to PC when compared to those that were not.[15] Furthermore, in the study of İnce *et al*., pro-inflammatory cytokines TNF-α and IL-1were found higher in the group of ischemia in the heart, whereas IL-10 of anti-inflammatory cytokines was found to be lower.[16] In a similar study, Xu *et al*. found that levels of pro-inflammatory cytokines in the PC group (TNF-α, IL-1, and IL-8) were lower in the lungs than in the IR group.[17] In our study, TNF-α and IL-1 levels were significantly lower in the IRPC group than in the IR group, but not statistically significant. In addition, IL-10 levels were significantly higher in the IRPC group than in the IR group, but not statistically significant.

Wang *et al*. detected a decrease in the infarct size by performing a PC in the heart of the rat isolated with morphine. It was suggested that the cardioprotective effect of PC might be mediated through kappa-opioid receptors and mitochondrial K-ATP channels.[18] Min *et al*. have administered 0.3, 3, and 30 μmol of morphine to the subjects to prove that opioid receptors use the ICAM-1 pathway.[3] We also gave our subjects 3 and 30 μmol of morphine during the reperfusion period and investigated their effects on IR injury. TNF-α and IL-1 levels in lung tissue were statistically significant between the IRPC3

group and the IR and IRPC groups. In addition, IL-10 level in lung tissue was statistically significant between the IRPC3 group and the IRPC group.

When we looked at the IL-1 level in the lung tissue, the difference between IRPC30 group and IR and IRPC groups were statistically significant. In addition, the difference between the IL-10 levels of the IRPC30 group and the IRPC group in lung tissue was significant. This showed that there was a statistically significant difference between pro-inflammatory and anti-inflammatory cytokines between the groups that received morphine and the groups that didn't. However, no differences in biochemical parameters were found in the groups in which morphine was administered at different doses.

Conclusion

IR injury is a pathophysiological process that is linked to most clinical processes such as acute lung injury, acute renal failure, shock, infection, and transplantation. The pathogenesis and molecular stages of IR damage, which have not been well understood, are a subject that is being studied extensively due to its frequency of appearance. In our study, we found statistically significant results when we compared the pro-inflammatory and anti-inflammatory cytokine values in lung tissue of experimental animals which received morphine in addition to the ischemic arresting protocol. In lung transplantation protocols, we think that morphine addition to PC will further reduce IR injury. There is a need to support this result with further studies.

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Conflicts of interest
There are no conflicts of interest.

References

1. Zimmerman BJ, Granger DN. Reperfusion injury. Surg Clin North Am 1992;72:65-83.
2. Mayfield KP, D'Alecy LG. Delta-1 opioid agonist acutely increases hypoxic tolerance. J Pharmacol Exp Ther 1994;268:683-8.
3. Min TJ, Kim JI, Kim JH, Noh KH, Kim TW, Kim WY, *et al.* Morphine postconditioning attenuates ICAM-1 expression on endothelial cells. J Korean Med Sci 2011;26:290-6.
4. Koç F, Erdem S, Altunkaş F, Ozbek K, Gül EE, Kurban S, *et al.* Ischemia-modified albumin and total antioxidant status in patients with slow coronary flow: A pilot observational study. Anadolu Kardiyol Derg 2011;11:582-7.
5. Ayan E, İlhan N. Effects of N-Acetylcystein in reperfusion injury after experimental lung ototransplantation model. Türk Göğüs Kalp Damar Cer Derg 2004;12:98-105.
6. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124-36.
7. Kin H, Zhao ZQ, Sun HY, Wang NP, Corvera JS, Halkos ME, *et al.* Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. Cardiovasc Res 2004;62:74-85.
8. Meldrum DR, Cleveland JC Jr. Mitchell MB, Sheridan BC, Gamboni-Robertson F, Harken AH, *et al.* Protein kinase C mediates Ca2(+)-induced cardioadaptation to ischemia-reperfusion injury. Am J Physiol 1996;271:R718-26.
9. Vinten-Johansen J, Zhao ZQ, Zatta AJ, Kin H, Halkos ME, Kerendi F, *et al.* Postconditioning – A new link in nature's armor against myocardial ischemia-reperfusion injury. Basic Res Cardiol 2005;100:295-310.
10. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, *et al.* Postconditioning the human heart. Circulation 2005;112:2143-8.
11. Tang Y, Pacary E, Fréret T, Divoux D, Petit E, Schumann-Bard P, *et al.* Effect of hypoxic preconditioning on brain genomic response before and following ischemia in the adult mouse: Identification of potential neuroprotective candidates for stroke. Neurobiol Dis 2006;21:18-28.
12. Zhao H, Sapolsky RM, Steinberg GK. Interrupting reperfusion as a stroke therapy: Ischemic postconditioning reduces infarct size after focal ischemia in rats. J Cereb Blood Flow Metab 2006;26:1114-21.
13. Bernaudin M, Nedelec AS, Divoux D, MacKenzie ET, Petit E, Schumann-Bard P, *et al.* Normobaric hypoxia induces tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain. J Cereb Blood Flow Metab 2002;22:393-403.
14. Hasturk A, Atalay B, Calisaneller T, Ozdemir O, Oruckaptan H, Altinors N, *et al.* Analysis of serum pro-inflammatory cytokine levels after rat spinal cord ischemia/reperfusion injury and correlation with tissue damage. Turk Neurosurg 2009;19:353-9.
15. Güneş T, Badak M, Kurtoğlu T, Karul A, Özkısacık EA, Dişcigil B. Effects of Ischemic Pre- and Post-conditioning in a Rat Model in Early Phase of Lower Extremity Ischemia-Reperfusion. Adnan Menderes Üniv Tıp Fakül Derg 2011;12:21-7.
16. İnce İ, Karapınar K, Özerdem G. The cardioplegia having n-acetylcysteine effects on myocardial protection. J Clin Anal Med 2015;6:61-4.
17. Xu B, Gao X, Xu J, Lei S, Xia ZY, Xu Y, *et al.* Ischemic postconditioning attenuates lung reperfusion injury and reduces systemic proinflammatory cytokine release via heme oxygenase 1. J Surg Res 2011;166:e157-64.
18. Wang J, Gao Q, Shen J, Ye TM, Xia Q. Kappa-opioid receptor mediates the cardioprotective effect of ischemic postconditioning. Zhejiang Da Xue Xue Bao Yi Xue Ban 2007;36:41-7.