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Experimental Study / Deneysel Çalışma

Pretreatment with carnosol in lung ischemia reperfusion-induced renal injury

Akciğer iskemi reperfüzyonunun tetiklediği böbrek hasarında karnosol ön koşullaması

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Background: This experimental study aims to investigate the protective effects of carnosol on kidney injury induced by lung ischemia/reperfusion (I/R).

Methods: Twenty-four New Zealand white rabbits were randomized into three groups. Group IR; lung I/R group (60 min ischemia-60 min reperfusion), group CIR; bolus injection of carnosol before lung I/R, and group S; sham group (pulmonary hilum was not clamped). Renal tissue myeloperoxidase (MPO) and intercellular adhesion molecule-1 (ICAM-1) levels were evaluated.

Results: Renal tissue MPO and ICAM-1 levels were significantly higher in the group IR compared to the group CIR and group S (p=0.021 and p=0.0001 respectively). No statistically significant difference in the parameters evaluated was detected between the group CIR and the group S.

Conclusion: Our study result suggests that lung I/R injury causes increased renal tissue MPO and ICAM-1 levels, which are related to activated neutrophil sequestration and carnosol may play a protective role against this kidney injury.

Keywords: Carnosol; ischemia reperfusion; lung; renal injury.

Amaç: Bu deneysel çalışmada, akciğer iskemi/reperfüzyonu (İ/R) ile indüklenen böbrek hasarı üzerine karnosolun koruyucu etkisi araştırıldı.

Çalışma planı: Yirmi dört Yeni Zelanda beyaz tavşanı üç gruba randomize edildi. Grup IR; akciğer İ/R grubu (60 dk. iskemi-60 dakika reperfüzyon), grup CIR; akciğer İ/R'si öncesi karnasol bolus enjeksiyonu ve grup S; plasebo grubu (pulmoner hilus klemplenmedi). Böbrek dokusu miyeloperoksidaz (MPO) ve interselüler adezyon molekülü -1 (ICAM-1) düzeyleri değerlendirildi.

Bulgular: Böbrek dokusu MPO ve ICAM-1 düzeyleri grup IR'de, grup CIR ve grup S ile karşılaştırıldığında, anlamlı olarak daha yüksek idi (sırasıyla, p=0.021 ve p=0.0001). Grup CIR ve grup S arasında ise ölçülen parametrelerde istatistiksel olarak anlamlı fark tespit edilmedi.

Sonuç: Çalışma sonuçlarımız akciğer İ/R'sine bağlı böbrek hasarında aktif nötrofil sekestrasyonu ile ilişkili MPO ve ICAM-1 düzeylerinin arttığını ve karnosolun bu hasarda koruyucu etkisi olabileceğini göstermektedir.

Anahtar sözcükler: Karnosol; iskemi reperfüzyon; akciğer; böbrek hasarı.

Lung transplantation has enjoyed increased success and became the mainstay of therapy for most end-stage lung diseases.[1,2] However, blood flow to the ischemic lung induces greater injury than the original ischemia itself, causing what is known as lung ischemia/reperfusion (I/R) injury.[1,3] This injury is one of the major complications of vascular and transplantation surgery[4] since it can lead to mortality and morbidity

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due to kidney injury in the postoperative period. Therefore, prevention of I/R injury to the kidneys is important for successful lung surgery. Most of the studies to date have evaluated the effect of kidney injury induced by renal tissue[5] as well as by different I/R models, including liver-related,[6] abdominal aorta-related,[6] and pneumoperitoneum-related I/R.[7] However, to the best of our knowledge, no previous study has investigated the effects of lung I/R injury on the kidneys.

A variety of inflammatory mediators, including myeloperoxidase (MPO) and intercellular adhesion molecule^{-1} (ICAM^{-1}), have been implicated in the systemic effects of I/R injury,[5,9-11] but because of the involvement of multiple factors in lung I/R injury, it has been difficult to achieve obvious protection by targeting any one single factor.[3] Myeloperoxidase and ICAM^{-1} levels, which are responses to I/R injury, increase substantially in the end-organ tissue.[5,9,12,13] Furthermore, the remote effects of I/R are most frequently observed in the renal and hepatic systems, and these can result in the development of systemic inflammatory response and multiple organ dysfunction syndromes.[14]

Carnosol, a major component of rosemary, is a phenolic diterpene that has been reported to exhibit antioxidant, anti-inflammatory,[15,16] anticancer,[17] and antiplatelet[18] effects. In addition, carnosol pretreatment attenuates liver injury induced by intestinal I/R, which may be attributable to its antioxidant effect.[16] However, there have been no reports regarding the effect of carnosol on kidney injury induced by lung I/R. Therefore, the primary aim of this experimental study was to investigate the protective effects of carnosol on lung IR-induced kidney injury. For this purpose, we produced I/R injury in an *in vivo* rabbit model and evaluated the levels of MPO and ICAM^{-1} in the remote tissue to check for I/R injury markers.

MATERIALS AND METHODS

Twenty-four New Zealand white rabbits weighing between 2150 g and 2500 g were used in this study. We received the approval of the Institutional Animal Care and Use Committee of Selcuk University ethics committee, and all of the animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals. The experiment was continued until there were eight surviving animals in each group over the course of the entire procedure as well as during the postoperative 60 minutes.

The animals were randomized into the three groups. The IR group received lung I/R, the carnosol-I/R (CIR) group was given a bolus injection of 3 mg/kg carnosol (Cayman Chemical Company, Ann Arbor, MI, USA) one hour before lung I/R, and the sham (S) group underwent the same surgical procedures except that the left pulmonary hilum was not clamped. The carnosol was dissolved in 10% dimethyl sulfoxide before intraperitoneal administration, and the dose administered was determined according to a previous study,[16] but was modified based on our preliminary experiments.

The animals were sedated with 50 mg/kg ketamine hydrochloride (Ketalar; Parke-Davis, Eczacibasi, Istanbul, Turkey) and 15 mg/kg xylazine (Rompun®, Bayer AG, Leverkusen, Germany) intramuscularly as a premedication. The ear artery and vein were then located, and arterial and peripheral venous lines were inserted. The systemic arterial pressure and heart rate were continuously monitored with the Datex Ohmeda Type F-CU8 anesthesia monitor (GE Healthcare Helsinki, Finland) throughout the procedure.

The trachea was then rapidly located in the neck via a vertical cervical incision, and a tracheostomy was performed. The trachea was then intubated with a 3 French intubation tube, and the animals were ventilated with a mechanical ventilator at a rate of 30 breaths/min with a tidal volume of 10 mL/kg, and an inspired oxygen fraction of 1.0.

Next, a left thoracotomy was performed under aseptic conditions. The left lung was then mobilized, the pulmonary hilum was dissected, and perivascular and peribronchial tissues were removed. Before inducing lung ischemia, 250 U/kg heparin was administered intravenously, and a 10-minute stabilization period was provided for the animals. The ischemic injury was induced in the IR and CIR groups by clamping the left pulmonary hilum, including the left main bronchus, vein, and artery, with a non-crushing microvascular clamp (ASSI, Westbury, NY) for 60 minutes, and reperfusion was achieved by removing the clamp for 60 minutes.[14]

At the end of the reperfusion period, the animals were euthanized by the intraperitoneal administration of 50 mg/kg thiopental (Pental Sodyum, İ.E. Ulagay ilaç Sanayi Türk A.Ş., İstanbul, Turkey). Afterwards, the left kidneys were harvested and stored at -80 °C for further analysis.

The supernatants of the homogenized tissue samples were analyzed to determine the tissue concentrations of MPO (Cusabio Biotech Co. Ltd., Wuhan, Hubei Province, P.R.China) and ICAM^{-1}

(Cusabio Biotech Co. Ltd., Wuhan, Hubei Province, P.R.China) using the enzyme-linked immunosorbent assay (ELISA) method via the Biotek ELx800™ Absorbance Microplate Reader (BioTek U.S., Winooski, VT, USA).

The standard curve concentrations used were 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, and 1.25 ng/mL for MPO and 4000 pg/mL, 2286 pg/mL, 914 pg/mL, 457 pg/mL, and 228.5 pg/mL for ICAM.

Statistical analysis

The data was analyzed using the SPSS for Windows version 15.0 software program (SPSS Inc., Chicago, IL, USA), and the results were expressed as mean ± standard deviation. In addition, the Kruskal-Wallis one-way analysis of variance (ANOVA) by ranks was used to detect differences between the groups, and statistical comparisons were made using the the Mann-Whitney U test. A p value of <0.05 was considered to be statistically significant.

RESULTS

Two animals died during the procedure due to surgical intervention. Renal tissue MPO levels and renal tissue ICAM^{-1} levels were presented in Figure 1 and Figure 2 respectively. The MPO and ICAM^{-1} levels were significantly higher in the IR group (p=0.021) compared with the CIR and S groups (p=0.0001 for both), and there was no statistically significant difference between the latter two groups.

DISCUSSION

Animal models have been useful in the development of new ideas for treatment, and they provide a bridge between patients and the laboratory. Animal lung I/R models can be divided roughly between studies using ventilated ischemia and anoxic ischemia. The most widely used models of pulmonary ischemia are clamping of the pulmonary artery, which leaves the bronchial circulation and venous return intact, and clamping of the hilum, which results in complete ischemia and anoxia. The latter method was used in this study.[14,19,20] Our results showed that 60 minutes of lung ischemia followed by 60 minutes of reperfusion can significantly increase the MPO and ICAM^{-1} levels in renal tissue.

In recent years, the main research goal in transplants has been to identify the main cellular and molecular bases of I/R injury.[11] The roles that neutrophils, alveolar macrophages, cytokines, and chemokines play in lung I/R injury as well as the importance of alterations in cell-death related pathways were nicely summarized in the study by den Hengst et al.[20] Furthermore, Parks and Granger[21] found that the results produced by three hours of ischemia and one hour of reperfusion were worse than those produced by four hours of ischemia without reperfusion. During reperfusion, endothelial cells generate oxygen free radicals. This causes the upregulation of ICAM^{-1} along with the activation of circulating neutrophils that adhere to the endothelium and release oxidants and proteases. This can result in microvascular injury.[9,22,23] Additionally, increased levels of adhesion molecules have been found in patients with organ damage.[22]

Some of the functions of ICAM^{-1} are that it can attract and/or activate leukocytes, potentiate small-vessel occlusion, and promote further production of inflammatory mediators.[5] In a study by Li et al.,[12] the ICAM^{-1} levels gradually increased in liver tissue after intestinal ischemia reperfusion, but then they decreased after reaching their peak between 12 and 24 hours. Cowley et al.[23] found elevated plasma levels of soluble adhesion molecules in patients with systemic inflammatory response syndrome and organ dysfunction. Moreover, Park and Han[5] reported that renal ischemia results in increased

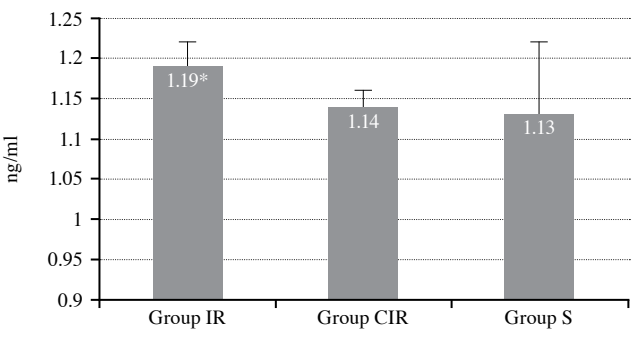

Figure 1. Renal tissue myeloperoxidase levels. * p=0.021 when compared with the S and CIR groups; IR: Ischemia reperfusion; CIR: Carnosol ischemia reperfusion; S: Sham.

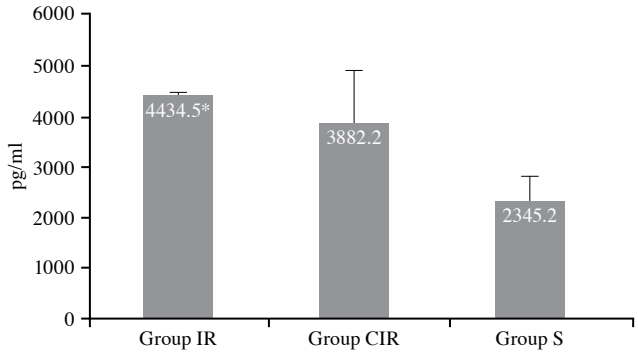

Figure 2. Renal tissue intercellular adhesion molecule-1 levels. * p=0.0001 when compared with the S and CIR groups; IR: Ischemia reperfusion; CIR: Carnosol ischemia reperfusion; S: Sham.

leukocyte infiltration and ICAM^{-1} expression in the kidney tissue of male mice. Myeloperoxidase, an enzyme located in leukocytes,[10] is one of the distinct indicators for the tissue infiltration of neutrophilic granulocytes, and MPO activity, which is a response to I/R injury, also increases substantially in end-organ tissue.[13]

Carnosol is a naturally occurring phytopolyphenol found in rosemary.[15,16,24] The main active compounds of this herb include caffeic acid, rosmarinic acid, ursolic acid, carnosic acid, and carnosol,[24] but approximately 90% of the total antioxidant activity is derived from the carnosol and carnosic acid.[24] The antioxidant, anti-carcinogenic, and anti-inflammatory properties of carnosol have been described using numerous cellular and animal models,[15,16,24,25] and it has been shown to reduce the proinflammatory leukotrienes in intact polymorphonuclear leukocytes, inhibit 5-lipoxygenase, and antagonize intracellular calmodulin-dependent protein kinase II (Ca^{2+}) mobilization.[25] Esme et al.[14] first evaluated the effects of lung I/R injury on remote organs (the lungs, liver, and heart, but not the kidneys). They constructed an *in vivo* I/R injury rabbit model and investigated its effect on MPO activity as an indicator of neutrophil recruitment in lung, heart, and liver tissue. Their results suggested that pulmonary I/R induces liver injury characterized by activated neutrophil sequestration and the release of significant amounts of reactive oxygen species (ROS). Our data showed that pulmonary I/R-induced kidney injury increased MPO activity when compared with the S group and that the renal ICAM^{-1} levels increased 1.89-fold in the IR group compared with the same group.

In conclusion, our findings suggest that neutrophil accumulation contributes to lung I/R-induced renal injury and that the protective effect of carnosol may be dependent, in part, on its inhibitory effect on tissue neutrophil infiltration. However, further histological, immunohistochemical, and gene studies should be carried out to clarify the protective role of carnosol against lung I/R-induced injury on the kidneys.

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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