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The effect of N-acetyl cysteine on serum glutathione, TNF-α and tissue malondialdehyde levels in the treatment of sepsis

Sepsis tedavisinde N-asetilsistein'in serum glutatyon, TNF-α ve doku malondialdehid düzeylerine etkisi

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BACKGROUND

The aim of this study was to investigate the effects of N-acetyl cysteine (NAC) on the levels of reactive oxygen species in sepsis.

METHODS

In this study, 30 Sprague-Dawley female rats weighing 180-200 g were used. Rats were randomized into three groups, each containing 10 rats, as follows: Group I: Sham, Group II: Sepsis and Group III: Sepsis+NAC. Group I underwent only laparotomy. In Groups II and III, sepsis was induced by cecal ligation and perforation (CLP) technique. NAC (20 mg/kg/day) was administered orally to Group III at 0, 8 and 16 hours. At the 24th hour, tissue and blood samples were taken for erythrocyte glutathione (GSH) and serum tumor necrosis factor (TNF)-α levels, histopathological determination, and lung, liver and kidney tissue malondialdehyde (MDA) analyses.

RESULTS

Group III was significantly different from the other groups with respect to erythrocyte glutathione, serum TNF-α and kidney MDA levels ($p<0.05$). There was no significant difference between the groups regarding liver MDA levels and histopathological parameters for lung, liver and kidney ($p>0.05$).

CONCLUSION

NAC treatment had beneficial effects on erythrocyte GSH, serum TNF-α, lung function, and kidney MDA levels in sepsis-induced rats. However, this beneficial effect was not confirmed as histopathological improvement. Further research is needed to prove the effect of NAC in sepsis treatment.

Key Words: Glutathione; N-acetyl cysteine; sepsis; tumor necrosis factor-α; malondialdehyde.

AMAÇ

Bu çalışmada, sepsis tedavisinde antioksidan bir ajan olan N-asetilsistein'in (NAC) serbest oksijen radikalleri düzeylerine olan etkileri araştırıldı.

GEREÇ VE YÖNTEM

Çalışmada ağırlıkları 180-200 gr arasında değişen 30 adet Sprague-Dawley cinsi dişi sıçan kullanıldı. Sıçanlar rastgele 10'arlı 3 gruba (Grup I: Sham, Grup II: Sepsis ve Grup III: Sepsis + NAC) ayrıldı. Grup I'e yalnızca laparotomi yapıldı. Grup II ve Grup III'de çekal ligasyon perforasyon (ÇLP) yöntemiyle sepsis modeli oluşturuldu. Grup III'e oral yoldan 0., 8. ve 16. saatte 20 mg/kg/gün NAC verildi. 24. saatte eritrosit glutatyon (GSH), serum TNF-α değerlerinin tayini için kan örnekleri ile histopatolojik inceleme ve doku malondialdehid (MDA) tayini için akciğer, karaciğer ve böbrek doku örnekleri alındı.

BULGULAR

Grup III'de TNF-α, eritrosit GSH ve böbrek doku MDA değerleri diğer gruplarla karşılaştırıldığında istatistiksel açıdan farklılık saptandı ($p<0,05$). Karaciğer doku MDA değerleri ve akciğer, karaciğer ve böbrek dokularının histopatolojik inceleme sonuçları açısından ise gruplar arasında farklılık bulunmadı ($p>0,05$).

SONUÇ

Sepsis oluşturulan sıçanlara verilen NAC tedavisinin eritrosit GSH, serum TNF-α düzeylerine, akciğer fonksiyonlarına, böbrek doku MDA seviyelerine olumlu etkileri saptandı. Buna karşılık bu olumlu etkinin histopatolojik düzelmeye yansımadığı görüldü. NAC'nin sepsis tedavisinde olası yararlı etkilerini ortaya koymak için yeni çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Glutatyon; N-asetilsistein; sepsis; tümör nekroz faktör-α; malondialdehid.

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Sepsis is a clinical condition requiring immediate treatment, and it still has high mortality and morbidity.[1] The pathophysiology of sepsis has become better understood, and mediators and cytokines taking part in the process and their mechanisms of action have been determined.[2] Specific agents like N-acetyl cysteine (NAC), NAME (N-nitro-L-arginine methyl ester), and desferrioxamine have been tried in some recent experimental studies.[3,4] NAC is a thiol compound with potent antioxidant and anti-inflammatory properties. It is also a well-known glutathione (GSH) precursor.[5,6] The aim of this study was to investigate the probable role of NAC in sepsis treatment and functional anomalies of the organs related to sepsis.

MATERIALS AND METHODS

This experimental study was carried out in Selcuk University Experimental Research and Training Center with approval of the ethics committee. A total of 30 female Sprague-Dawley rats weighing 180-200 g were used in the study. The rats were fed with unlimited rat feed and water under standard laboratory conditions before the study. They were allowed to take only water for 12 hours before and after the operation. Only one rat died at the 20th hour due to the severity of the sepsis. No deaths were observed in the other groups. The rats were randomly divided into three groups of 10 rats each.

Group I (Sham group): Anesthesia and operative procedure were applied on the subjects but cecal ligation perforation was not performed.

Group II (Sepsis group): Sepsis was created using the cecal ligation and perforation (CLP) method.

Group III (NAC group): Sepsis was created using the CLP method. NAC (20 mg/kg/day) was administered to the rats perorally using the feeding tube in three equal doses at 0, 8 and 16 hours after the operation.

General anesthesia was applied to the rats using subcutaneous ketamine HCl (50 mg/kg) and xylazine HCl (15 mg/kg). Once the subjects were immobilized on the operation table in the supine position and the abdominal skin was shaved completely, laparotomy was performed through a 2 cm midline incision. The CLP model was chosen to create sepsis.[7] The cecum was isolated following laparotomy, and the ascending colon was stroked downward so as to fill the cecum with feces. The cecum was ligated below the ileocecal valve using 3/0 silk thread, and the ventral side was perforated twice using No. 18 venous cannula needle. The abdomen was then closed with continuous suture as two layers using 3/0 silk thread. CLP was not applied in the sham group, and only the cecum was explored. The rats were monitored in rooms at a temperature of 22°C, with controlled humidity, light and heat, and their drugs were administered at the designated hours. The rats were allowed to take standard rat feed and drinking water after the 12th postoperative hour. All the rats were sacrificed 24 hours after the operation.

Blood samples drawn through cardiac puncture were transferred to the Selcuk University Biochemistry Laboratory in ice packs. The blood samples were centrifuged at 3000 cycles/minute for 5 minutes in the frigorific centrifuge prepared formerly and the plasma and serum were separated. The samples were transferred into three distinct Eppendorf tubes and stored at -70°C until the time of analysis as they were considered to be studied at different times. Determination of the erythrocyte GSH and serum tumor necrosis factor (TNF)-α was made from the erythrocyte hemolysate and serum obtained from centrifugation using proper kits.

Tissue malondialdehyde (MDA) levels were determined using the manual method after obtaining the hepatic, pulmonary and renal tissue samples. Tissue samples of 1 g were obtained from the lung, liver and kidney and sent to the pathology laboratory in 10% formol solution for histopathological examination. Paraffin blocks were prepared and 5 microns of sections were obtained and stained with hematoxylin-eosin. The specimens were examined under light microscopy at X40 magnification. Examinations were performed by pathology specialists. Pathological findings were scored semiquantitatively and interpreted as follows: 0: normal, +1: mild, +2: moderate, +3: severe, and +4: excessive.[8] Alveolar septal thickening, congestion, hemorrhage, and presence and severity of pulmonary infiltration were evaluated in the histopathological sections of the lung tissue; congestion, hydropic degeneration, focal necrosis, and presence and severity of polymorphonuclear leukocytes (PMNL) were evaluated in the histopathological sections of the liver tissue; and congestion, pericapsular inflammation, tubular vacuolization, and presence and severity of PMNL were evaluated in the histopathological sections of the kidney tissue.

Statistical Analysis

The groups were analyzed as tables by calculating the mean and standard deviation values. Statistical analysis was performed using the SPSS for Windows 13.0 program. Inter- group comparisons were made using the one-way variance analysis (ANOVA). The Tukey HSD test was used as the post hoc test. The chi-square and the Kruskal-Wallis tests were used for analysis of histopathological score data in the liver, lung and renal tissues, and the Mann-Whitney U test with Bonferroni correction was applied for significant data. P values of <0.05 were considered statistically significant.

RESULTS

The decrease in serum TNF-α values and the increase in erythrocyte GSH values in the NAC group compared to the sepsis group were statistically significant ($p<0.05$), but the decrease in liver MDA values was not statistically significant ($p>0.05$). The decrease in renal MDA values in the NAC group was found to be statistically significant compared to the sepsis group ($p<0.05$) (Table 1). In the histopathological examination of the tissues, while the decrease in PMNL in lung tissue in the NAC group was found to be statistically significant compared to the sepsis group ($p<0.05$), it was not statistically significant in terms of alveolar septal thickening, congestion and hemorrhage in lung tissue ($p<0.05$) (Table 2). Histopathological improvement in the liver and renal tissues was not found to be statistically significant in the NAC group compared to the sepsis group ($p>0.05$) (Tables 3 and 4).

DISCUSSION

In sepsis, cytokines like TNF-α, interleukin-1 (IL-1) beta and IL-6 released after infection play important roles in the initiation of the inflammatory process.[9] Useful effects of several vasodilator agents with anti-inflammatory properties have been reported in the treatment of sepsis. In particular, an antioxidant agent, NAC, has been shown to improve cardiac performance and liver functions via improving the hepatosplanchnic perfusion.[10] Tang et al.[11] demonstrated that treatment with a GSH precursor, NAC, had prevented Group B streptococcus growth in the lung, liver and spleen in an experimental rat model induced with ethanol. In the study of Hsu et al.[12] in rats, they concluded that NAC treatment suppressed the release of inflammation markers TNF-α, IL-6 and IL-10 in organ insufficiency related to endotoxin shock. It was claimed that liver, heart and kidney injuries were minimized with this useful effect of NAC. In this experi-

Table 1. Serum TNF-α, erythrocyte GSH and tissue MDA values of the groups

	TNF-α (pg/ml)	GSH (μM)	Liver MDA (nmol/ml)	Kidney MDA (nmol/g protein)
Group I	8.07± 3.98	156.14±35.35	2.28±0.66	2.21±0.39
Group II	19.03±7.90 [a]	92.13±21.16 [a]	4.33 ±1.24 [a]	4.26±1.47 [a]
Group III	7.85±4.99 [b]	147.98±53.65 [b]	2.94 ±0.61	2.76±0.32 [b]

a: $p<0.05$ when compared to Group I; b: $p<0.05$ when compared to Group II.
MDA: Malondialdehyde; GSH: Glutathione; TNF-α: Tumor necrosis factor-alpha.

Table 2. Lung histopathological scores of the groups

	PMNL infiltration	Alveolar septal thickness	Congestion	Hemorrhage
Group I	0.40±0.51	0.90±0.73	0.30±0.48	0.50±0.70
Group II	2.78 ±0.66 [a]	2.89±1.05 [a]	2.22±0.66 [a]	2.44±1.13 [a]
Group III	1.60±0.51 [b]	1.77 ±1.01	1.36±0.87	1.40±0.51

a: $p<0.05$ when compared to Group I; b: $p<0.05$ when compared to Group II; PMNL: Polymorphonuclear leukocytes.

Table 3. Liver histopathological scores of the groups

	PMNL infiltration	Hydropic degeneration	Congestion	Focal necrosis
Group I	0.20±0.42	0.00±0.00	0.50±0.52	0.00±0.00
Group II	1.56±0.52 [a]	1.33±0.50 [a]	2.78±0.83 [a]	1.44±0.52 [a]
Group III	1.40±0.51	1.30±0.48	1.70±0.67	1.50±0.52

a: $p<0.05$ when compared to Group I; b: $p<0.05$ when compared to Group II; PMNL: Polymorphonuclear leukocytes.

Table 4. Kidney histopathological scores of the groups

	PMNL infiltration	Pericapsular inflammation	Congestion	Tubular vacuolization
Group I	0.10±0.31	0.00±0.00	0.20±0.42	0.00±0.00
Group II	1.78±0.83 [a]	1.78±0.83 [a]	1.44±0.52 [a]	1.56±0.52 [a]
Group III	1.60±0.696	1.10±10.56	1.40±0.51	1.30±0.67

a: $p<0.05$ when compared to Group I; b: $p<0.05$ when compared to Group II; PMNL: Polymorphonuclear leukocytes.

mental study, the decrease in the serum TNF-α levels in the NAC group was found to be statistically significant compared to the sepsis group.

In the study of Ortolani et al.,[13] they used GSH and NAC for prevention of free radical injury in the early phase of septic shock. High-dose NAC treatment added to GSH was found to significantly decrease the perioxidative stress in early septic shock patients.

In the experimental study of Vassilev et al.,[14] they investigated the effects of NAC on gas exchange and metabolism in the systemic, pulmonary and hepatosplanchnic areas in endotoxemia, which is a condition similar to sepsis in humans. In that study, NAC treatment was found to increase the GSH concentration. On the other hand, NAC was found to be ineffective on oxygen exchange and metabolism in the systemic, pulmonary and hepatosplanchnic areas. In this study, only erythrocyte GSH values were found to decrease secondary to oxidative stress in the sepsis group. Elevations in erythrocyte GSH values in the NAC group were found to be statistically significant in terms of the efficacy of treatment.

Victor et al.[15] investigated the effects of NAC on the reducing condition of peritoneal macrophages and lymphocytes in a fatal endotoxic shock model experimentally created in rats. In the study, 150 mg/kg intraperitoneal NAC treatment administered 30 minutes after the lipopolysaccharide (LPS) procedure was found to reduce reactive oxygen species, TNF-α, MDA levels, and the oxidized reduced GSH rate. NAC was shown to prolong the survival of rats. In this study, NAC treatment was found to have a positive effect on renal MDA values.

In the study of Cetinkaya et al.[16] investigating the effectiveness of NAC in oxidative liver injury due to methotrexate, NAC treatment was found to increase GSH levels and decrease the MDA and myeloperoxidase (MPO) levels. In light of these data, it was suggested that NAC could be used as a therapeutic agent in the prevention of hepatotoxicity in patients under methotrexate treatment. In this study, the finding of NAC treatment being ineffective on liver MDA was determined to be related to the insufficient dose and short duration. We suggest that better outcomes can be obtained in further studies with high doses and longer durations.

In rats with sepsis-induced experimental acute lung and brain injury, a significant decrease was detected in interstitial edema, alveolar injury, inflammatory cell infiltration, and vascular congestion in the group treated with NAC and silymarin.[17] In another experimental sepsis model created by endotoxin administration to rats, it was found that histopathological findings such as alveolar and interstitial hemorrhage and edema and leukocyte infiltration improved in groups treated with high doses of NAC.[18] In our study, in the histopathological examination of the lungs, the increase in PMNL was found to have significantly decreased in the NAC group compared to the sepsis group. The improvements in congestion, hemorrhage and septal thickening in the lung tissue and the histological improvements in the kidney and the liver were not statistically significant. The ineffectiveness of NAC treatment on congestion, hemorrhage and septal thickening and the histopathological findings in the kidney and liver was considered to be related to low doses of NAC (20 mg/kg/day).

In conclusion, this study demonstrated that low doses of NAC administration converted sepsis-induced oxidative injury by positively affecting the erythrocyte GSH and serum TNF-α levels, pulmonary functions and renal tissue MDA levels. On the other hand, this positive effect was seen to not be reflected in histopathological improvement. Further studies with different doses of NAC are needed to put forth the other possible positive effects in sepsis treatment.

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