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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

Protective Effects of Apocynin on Cecal Ligation and Puncture-Induced Lung and Renal Injuries

Apokininin Çekal Ligasyon ve Ponksiyon ile İndüklenen Akciğer ve Böbrek Hasarına Karşı Koruyucu Etkileri

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ABSTRACT

Aim: The possible beneficial properties of Apocynin against cecal ligation and puncture (CLP) induced lung and renal injuries were investigated in rats.

Material and Method: 32 Wistar Albino male rats were randomized as: group I (sham), group II (CLP), group III (CLP+Apocynin 20 mg/kg), and group IV (CLP+Apocynin 50 mg/kg). CLP process was carried out under anesthesia conditions. Apocynin was administered intraperitoneally prior to the CLP model. The lung and renal tissues were excised following the experiment. Biochemical analyzes were done.

Results and Conclusion: TNF-α, OSI, IL-1β, TOS, MDA levels and MPO activity elevated but SOD and TAS values declined in CLP group compared to sham group. On the other hand, SOD and TAS levels increased while MPO activity, TNF-α, IL-1β, TOS, OSI and MDA levels declined due to Apocynin treatments. As a conclusion, Apocynin is an effective agent against CLP-induced lung and renal injuries.

Keywords: Apocynin, cecal ligation and puncture, lung, renal, rat

ÖZ

Amaç: Sıçanlarda Apokinin'in, çekal ligasyon ve ponksiyona (CLP) bağlı akciğer ve böbrek hasarına karşı olası yararlı özellikleri araştırıldı.

Gereç ve Yöntem: 32 Wistar Albino erkek sıçan randomize olarak grup I (sham), grup II (CLP), grup III (CLP+Apokinin 20 mg/kg) ve grup IV (CLP+Apokinin 50 mg/kg) olarak düzenlendi. CLP işlemi anestezi koşulları altında gerçekleştirildi. Apokinin, CLP modelinden hemen önce intraperitonal olarak uygulandı. Akciğer ve böbrek dokuları deneyin ardından eksize edildi. Biyokimyasal analizler yapıldı.

Bulgular ve Sonuç: Sham grubuna kıyasla CLP grubunda TNF-a, OSI, IL-1B, TOS, MDA düzeyleri ve MPO aktivitesi yükselirken SOD ve TAS değerleri azaldı. Öte yandan, Apokinin uygulaması sonucunda MPO aktivitesi, TNF-α, IL-1β, TOS, OSI ve MDA seviyeleri düşerken SOD ve TAS düzeyleri artmıştır. Sonuç olarak, Apokinin CLP kaynaklı akciğer ve böbrek hasarına karşı etkili bir ajandır.

Anahtar Kelimeler: Apokinin, çekal ligasyon ve ponksiyon, akciğer, böbrek, sıçan

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INTRODUCTION

Sepsis is a global health problem in intensive care units (1). It affects several patients and demonstrates high mortality rates (2,3). Lungs are among the organs mostly expose to undesirable effects of sepsis (4). Acute lung injury (ALI) accompanies to sepsis. When the prognose of sepsis gets worse, acute respiratory distress syndrome (ARDS) may exist (5). Kidneys also expose to septic injury such as lungs. During sepsis, acute lung injury (ALI) and acute kidney injury (AKI) lead to high mortality rates (6,7). Systemic response may occur due to various reasons such as raised inflammatory mediators and uncontrolled infections (8). Sepsis related ALI and ARDS involve elevation in tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and other several proinflammatory cytokines (9-11). Inflammatory response includes the stimulation of cytokines which leads to an elevation in reactive oxygen species (ROS) production (12).

Apocynin (Apoc) is a medicine herb that is obtained from *Picrorhiza kurroa* (13,14). It is preferred as nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (NOX2) inhibitor (14,15). Apoc demonstrated anti-inflammatory activity in both cell and animal inflammation models (13). Here, Apoc was examined to mitigate the oxidative damage in cecal ligation and puncture (CLP)-induced polymicrobial sepsis model.

MATERIALS AND METHODS

Ethical Approval, Animals, and Drugs

Allowance of the study was provided by Atatürk University Experimental Animal Ethics Committee (protocol no:30.03.2018/64) and carried out at Experimental Animals Research and Application Center of Atatürk University. 32 Wistar Albino male rats each nearly weighing 240-270 g, were obtained from the same center. They were housed in laboratory medium with appropriate cages, humidity, temperature and light/darkness conditions. Standard rat feed and tap water were provided to the animals. They were prohibited of food 12 hours prior to the experiment, but had permission to drink water. Thiopental sodium was preferred for anesthesia and it was purchased from Ulagay, İstanbul, Turkey. Apoc was provided by Sigma-Aldrich Co.

Experimental Animals and Experimental Design

Prior to experimental process, the rats were fixed in supine position, abdominal areas were prepared by shaving and disinfecting with povidone-iodine. Anesthesia was administered intraperitoneally (i.p.). The rats were randomly assigned to 4 groups: Group I (Sham group, n=8): Abdominal area was opened with a 2 cm incision to reach peritoneum and then sutured with a 3.0 silk suture but no more intervention was done. Group II (CLP group, n=8): Following the steps in group I, it was reached to peritoneum for the isolation of cecum. Cecum was ligated up to 2 cm distal and the ileocecal valve was pierced via 18-gauge needle (4 holes). Following the replacement of cecum, the incision was sutured. Group III (CLP+Apoc 20 mg/kg group, n=8) and Group IV (CLP+Apoc 50 mg/kg group, n=8): Apoc was administered i.p. at 20 and 50 mg/kg doses just before the CLP model precess as described in group II. The rats were fasted postoperatively, but water was allowed ad libitum for 18 hours until they were sacrificed.

Biochemical Measurements

Renal and lung tissues were prepared as 100 mg for each sample. They were homogenized via phosphate buffer solution (PBS) and centrifuged to obtain supernatant. The supernatants were kept at -80 °C. Determination of MDA is the measurement of product which occurs due to reaction between MDA and thiobarbituric acid (16). TAS and TOS parameters were evaluated via ELISA kits (Rel Assay Diagnostics). OSI is the ratio of TOS to TAS and was found as: OSI=[(TOS, μmol/L)/(TAS, mmol/L)×10]. Oxidation product of MPO and o-dianisidine is used to find out MPO activity (17). Formazan dye level is inversely proportional with SOD value and preferred for the assessment of SOD level (18). TNF-α and IL-1β levels were examined with appropriate kits (Elabscience, Wuhan, China).

Statistical analysis

Results have been determined by One-way ANOVA. Tukey test was used for pairwise comparisons of groups. All the results have been presented as mean±SD. The differences have been approved significant when $p<0.05$.

RESULTS

Effect of Apoc on oxidative stress markers in lung tissues

Lung biochemical parameters in all groups were shown in **Table 1.** TOS, MPO, MDA and OSI levels elevated while TAS and SOD concentrations declined significantly in CLP group compared to sham group. With Apoc 20 mg/kg treatment, these parameters conversely changed except for MPO level when compared with CLP group. Apoc 50 mg/kg treatment changed significantly all biochemical markers compared with CLP group.

Effect of Apoc on oxidative stress markers in kidney tissues

Renal biochemical parameters in all groups were presented in **Table 2.** TOS, MPO, MDA and OSI levels increased while TAS and SOD values diminished significantly in CLP group compared to sham group. Apoc 20 mg/kg treatment reversed these parameters significantly except for SOD levels compared with CLP group. Apoc 50 mg/kg treatment changed all biochemical markers significantly compared with CLP group.

Table 1. Effects of Apoc treatment on biochemical parameters in CLP-induced lung injury

Groups (n=8)	Sham	CLP	Apoc 20mg/kg	Apoc 50 mg/kg
TAS (mmol/L)	0,55±0,04	0,23±0,01[a]	0,36±0,04[b]	0,47±0,06[b]
TOS (μmol/L)	6,99±0,43	8,51±0,21[a]	7,30±0,74[b]	7,23±0,50[b]
OSI (arbitrary unit)	1,26±0,11	3,64±0,32[a]	2,00±0,21[b]	1,54±0,21[b]
SOD (U/mg protein)	394,18±49,32	247,09±30,72[a]	310,75±33,77[b]	368,68±34,94[b]
MPO (U/g protein)	187079,67±44824,96	440527,32±34577,78[a]	314559,74±50127,98[b]	210031,82±56425,00[b]
MDA (μmol/g tissue)	57,62±4,65	75,25±16,75[a]	63,70±8,50	60,69±7,37[c]

[a]p < 0.001 compared to sham group. [b]p< 0.001 and [c]p<0.05 compared to CLP group

Table 2. Effects of Apoc treatment on biochemical parameters in CLP-induced kidney injury

Groups (n=8)	Sham	CLP	Apoc 20mg/kg	Apoc 50 mg/kg
TAS (mmol/L)	1,85±0,12	1,12±0,17[a]	1,67±0,24[b]	1,88±0,21[b]
TOS (μmol/L)	6,43±0,60	7,80±0,87[a]	6,83±0,33[c]	6,45±0,63[b]
OSI (arbitrary unit)	0,34±0,03	0,70±0,09[a]	0,41±0,08[b]	0,34±0,05[b]
SOD (U/mg protein)	237,77±38,85	162,91±37,48[d]	228,10±63,2[b]	238,15±69,94[c]
MPO (U/g protein)	380829,34±58111,42	524799,67±36517,17[a]	422155,24±33192,91[b]	370196,81±90373,47[b]
MDA (μmol/g tissue)	72,25±10,00	98,69±9,21[a]	75,63±9,26[b]	73,32±12,14[b]

[a]p < 0.001 and [d]p<0.05 compared to sham group. [b]p< 0.001 and [c]p<0.05 compared to CLP group

Effect of Apoc on inflammatory markers in lung tissues

The effect of Apoc on pro-inflammatory markers in treatment group group were shown in **Figure 1**. The levels of TNF-α and IL-1β were significantly elevated in CLP group. In the groups that received pretreatment with Apoc, there was a significant amelioration of the increased inflammatory mediators (TNF-α and IL-1β) in a dose-dependent manner.

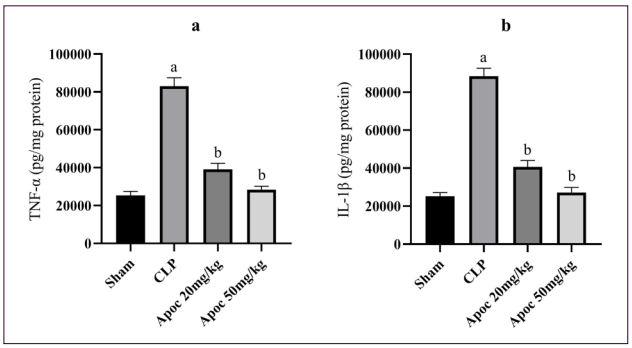

Figure 1. Effects of Apoc treatment on TNF-α and IL-1β levels in CLP-induced lung injury. All values are the mean±SD. [a]p<0.001 versus sham group and [b]p<0.001 versus CLP group.

Effect of Apoc on inflammatory markers in kidney tissues

The levels of the cytokine TNF-α and IL-1β in kidney tissues were dramatically increased in CLP group (**Figure 2**). In groups treated with Apoc (20 mg/kg and 50 mg/kg), there was a significant amelioration of the increased inflammatory mediators (TNF-α and IL-1β) in a dose-dependent manner.

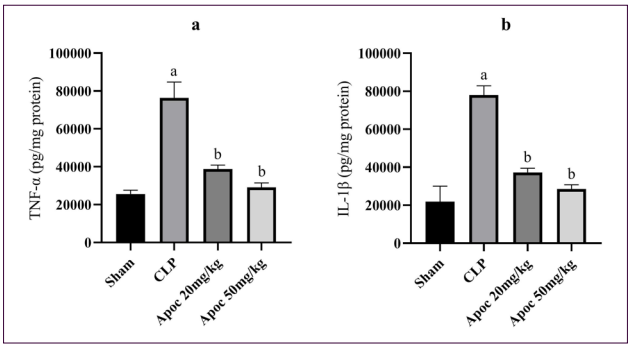

Figure 2. Effects of Apoc treatment on TNF-α and IL-1β levels in CLP-induced kidney injury. All values are the mean±SD. [a]p<0.001 versus sham group and [b]p<0.001 versus CLP group.

DISCUSSION

Sepsis is a critical health condition and arises from bacterial infections. It leads to uncontrolled inflammatory response which ends up with multiple organ failure and even death (19). ALI accompanies CLP-induced sepsis cases and it is a primary health problem to be coped (20,21). Infection-based respiratory failure results in death about in 2.3 million patients annually which takes the 6th place among the cause of all deaths (22). Sepsis is the primary cause of AKI and ALI. Various studies showed that sepsis related ALI and AKI have a multifactorial and complex pathophysiology in which inflammatory pathways, oxidative stress play role (23,24).

CLP method is commonly preferred to create a sepsis model (25-29). CLP results in peritonitis, bacteremia, shock, organ failure and even death (30). Sepsis leads to sustained massive inflammation and inhibition of

immune response (31,32). During sepsis, endothelial injury leads to ROS generation and inflammation (33). Excessive elevation of inflammatory mediators leads to sepsis in which hypotension and multiple organ failure is typical (34,35). Increase in TNF-α, IL-1β and other cytokines aggravate the inflammatory response (8,36). Early examples of this models showed that TNF-α, IL-1β and some other cytokines were increased (37,38). MPO is located in neutrophils and an increase in MPO activity reflects neutrophil activation (39). MPO activity also elevates in ischemic conditions or excessive tissue injury such as sepsis (26,40,41). MPO activity is admitted as a pulmonary neutrophil accumulation index (42). Here, MPO activity elevated in CLP group at both tissue samples. When Apoc treatment is considered for MPO activity, for renal tissue samples, MPO activity declined in both Apoc treatment groups. In lung tissue samples, there was a significant decrease with high Apoc level. MDA occurs due to lipid peroxidation and increases in case of inflammation (43). Sepsis related CLP caused elevation in lung tissue MDA level in various studies (44,45). In current study, MDA level increased in both tissues of CLP group, and there was a significant decrease in treatment groups. SOD scavenges superoxide anions. To evaluate the level of oxidative stress, MDA and SOD are usually analyzed simultaneously (46). Oxidative stress can be determined through TOS and TAS measurement (47,48). In current study, decrease in TAS level, increase in TOS and OSI levels were observed in CLP group when compared to sham group and these parameters returned to normal values with Apoc treatment in both tissue samples.

Various agents performing anti-inflammatory and antioxidant features were examined in sepsis models (49). Antioxidants may deactivate ROS and thus they may take role in therapies multiple organ dysfunction with sepsis (50).

Apoc is a NOX2 inhibitor in several cells (51). Apoc is used in the treatment of various oxidative stress and inflammatory related diseases (13). Previous researches have established that Apoc organizes the oxidative balance and declines the oxidative molecule levels (52,53). Previous research findings into Apoc study has shown that liver inflammation is reduced with Apoc treatment (53).

Thus far, literature doesn't involve studies about Apoc with CLP-induced lung and renal injuries. Here, it was investigated Apoc and its effects on ALI and AKI injuries induced by CLP sepsis model.

CONCLUSIONS

Apoc provides a protection against lung and renal injuries arising from CLP-induced sepsis. Treatment with Apoc at different doses reduces lung and renal damages in experimental animals exposed to CLP-induced sepsis model.

ETHICAL DECLARATIONS

Ethics Committee Approval: Allowance of the study was provided by Atatürk University Experimental Animal Ethics Committee (protocol no:30.03.2018/64) and carried out at Experimental Animals Research and Application Center of Atatürk University.

Informed Consent: None.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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