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Özgün Araştırma

Reno-Protective Effects of Brusatol Against Renal Ischemia Reperfusion Injury

Brusatol'ün Renal İskemi Reperfüzyon Hasarına Karşı Renoprotektif Etkileri

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ABSTRACT

Objective:

The aim of this study was to determine the potential beneficial effects of brusatol treatment on oxidative kidney injury induced by bilateral renal ischemia reperfusion (RIR) method.

Material and Method:

In the existing study, experimental animals were randomly assigned to 4 groups as sham, RIR, Dimethyl Sulfoxide (DMSO) and brusatol groups. Sham group; the back region was opened by incision and then sutured but no ischemia reperfusion (IR) model was established. In RIR group, 1 hour of ischemia following 24 hours of reperfusion was formed. In DMSO group, 0.3 ml, 1% DMSO was administered intraperitoneally. Then IR model was carried out as told in RIR group. In brusatol group, brusatol was applied intraperitoneally as 0.5 mg/ml for each rat every other days for 10 days before the experiment. The last dose was administered 30 minutes before reperfusion and IR was fulfilled as depicted in RIR group. Following reperfusion period, rats were immolated and renal tissues were isolated. Tumor Necrosis Factor alpha (TNF-α), Malondialdehyde (MDA), Interleukin 1beta (IL-1 β), Oxidative Stress Index (OSI), Total Oxidant Status (TOS), Total Antioxidant Status (TAS) levels, Superoxide Dismutase (SOD) and Myeloperoxidase (MPO) activity in tissue samples were analyzed using biochemical methods.

Results:

TNF-α, MDA and IL-1β levels, OSI, TOS and MPO values were significantly raised but TAS and SOD levels were declined in RIR and DMSO groups compared to sham group. On the other side, TAS and SOD increased while OSI and TOS values, activity of MPO and TNF-α, MDA and IL-1β levels were significantly reduced in brusatol+I/R group due to brusatol therapy compared to sham and DMSO groups.

Conclusion:
It has been thought that brusatol alleviated oxidative damage thanks to its antioxidant and anti-inflammatory properties. Consequently, brusatol demonstrated protective effects against RIR-induced oxidative kidney injury in rats.

Key Words:
Brusatol, Renal Ischemia Reperfusion, Oxidative stress, Rat

ÖZ
Amaç:
Bu çalışmanın amacı, brusatol tedavisinin bilateral renal iskemi reperfüzyon (RIR) yöntemi ile indüklenen oksidatif böbrek hasarı üzerindeki potansiyel yararlı etkilerini belirlemektir.

Gereç ve Yöntem:
Mevcut çalışmada, deney hayvanları rastgele sham, renal iskemi reperfüzyonu (RIR), Dimetil sülfoksit (DMSO) ve brusatol grupları olarak 4 gruba ayrıldı. Sham grubu; arka bölge insizyon ile açıldı ve sonra sütüre edildi ancak iskemi reperfüzyon (IR) modeli oluşturulmadı. RIR grubunda 1 saatlik iskemiyi takiben 24 saatlik reperfüzyon oluşturuldu. DMSO grubunda, her sıçan için 0,3 ml %1 DMSO intraperitonal olarak uygulandı. Daha sonra RIR grubunda anlatıldığı gibi IR modeli uygulandı. Brusatol grubunda brusatol, deneyden önce 10 gün boyunca her gün her bir sıçan için 0,5 mg/ml olarak intraperitonal olarak uygulandı. Son doz reperfüzyondan 30 dakika önce uygulandı ve IR, RIR grubunda gösterildiği gibi gerçekleştirildi. Reperfüzyon döneminden sonra sıçanlar hareketsiz hale getirildi ve böbrek dokuları izole edildi. Doku örneklerinde Tümör Nekroz Faktör Alfa (TNF-α), Malondialdehit (MDA), İnterlökin 1Beta (IL-1β), Oksidatif Stres İndeksi (OSI), Total Oksidan Seviyesi (TOS), Total Antioksidan Seviyesi (TAS) düzeyleri, SOD ve MPO aktivitesi biyokimyal metodlar kullanılarak analiz edildi.

Bulgular:
TNF-α, MDA ve IL-1β düzeyleri, OSI, TOS ve MPO değerleri anlamlı olarak yükselmiş, ancak TAS ve SOD düzeyleri sham grubuna göre RIR ve DMSO gruplarında azalmıştır. Öte yandan TAS ve SOD artarken OSI ve TOS değerleri, MPO ve TNF-α, MDA ve IL-1β düzeylerinin brusatol+I/R grubunda sham ve DMSO gruplarına göre brusatol tedavisine bağlı olarak anlamlı düzeyde azaldığı görülmüştür.

Sonuç:
Brusatolün sahip olduğu antioksidan ve anti-enflamatuar özellikleri sayesinde oksidatif hasarı hafifletmiş olduğu düşünüldü. Sonuç olarak, brusatolun sıçanlarda RIR kaynaklı oksidatif böbrek hasarına karşı koruyucu etkiler gösterdiği söylenebilir.

Anahtar Kelimeler:
Brusatol, Renal İskemi Reperfüzyonu, Oksidatif stres, Sıçan

INTRODUCTION
Ischemia reperfusion (IR) typically results in acute kidney injury (AKI) which causes high morbidity and mortality (1-2). AKI is the rapid deterioration in renal functions stemming from numerous situations such as IR or toxic conditions (3). It is characterized with sudden (within hours) decline in renal functions and structural damage of kidneys (4). IR is the most common AKI reason which generally occurs after shock, partial nephrectomy, severe trauma and kidney transplantation (5-7). Tissue injury is resulted from renal hypoxia that causes inflammatory signal activation, reactive oxygen species (ROS) generation, endothelial dysfunction and apoptosis. Restoring the blood flow (reperfusion) of an ischemic region fairly increases the tissue injury (8). Free radicals and ROS enhance IR injury. 50% of all AKI cases include renal IR (RIR) injury (9). Total oxidant status (TOS) and myeloperoxidase (MPO) are important parameters that increase IR-induced tissue damage. Oxidative stress index (OSI) represents TOS to total antioxidant status (TAS) ratio and it is an effective parameter for evaluating the tissue oxidative stress degree (10). MPO enzyme, found in neutrophils, mainly described as a marker for neutrophil infiltration (11). Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are antioxidant enzyme samples and act on tissue protection against oxidative injury (12). Therefore, interventions such as anti-inflammatory and antioxidant agents against RIR injury would be beneficial (13, 14).

Brucea javanica fruit and seed oil have been used in Chinese medicine (15). Brusatol, a novel natural herb obtained from Brucea javanica, has also been widely used to help treating tumors, inflammation and malaria (16). It has insecticide, antitumor, antiviral, antimalarial and anti-inflammatory features (16). In this research, we intended to evaluate the effect of brusatol in order to ease the oxidative damage in RIR model.

MATERIALS and METHODS
Experimental Animals and Ethical Approval
Ethical approval was obtained through Atatürk University Experimental Animals Local Ethics Committee (28.03.2019/68). Experimental Animal Research and Application Center of Atatürk University was preferred for obtaining the animals and maintaining the experimental processes. We declare that the study was carried out in accordance with Research and Publication Ethics. Medium for the animals were planned as appropriate laboratory conditions and rats were housed in regular cages. Providing of standard pellet and tap water to the rats was ended twelve hours prior to the experiment. We also declare that this study was conducted in accordance with Research and Publication Ethics.

Groups and Ischemia Reperfusion Model
Povidone iodine was used for disinfection and all surgical processes were applied under anesthesia of 10 mg/kg i.p. xylazine hydrochloride (Rompun®, Bayer, Istanbul) and 50 mg/kg i.p. ketamine (Ketalar®, Pfizer, Istanbul) (17). 32 Sprague Dawley male rats weighing 230±5g were randomized to 4 groups. Sham group; back region of rats was prepared for incision by shaving and cleaning steps. 1-2 cm size of incision

was followed via repairing by 3/0 suture but no other processes. Renal IR (RIR) group; same procedures of sham group were carried out and the veins and arteria of bilateral kidneys were fixed with atraumatic clamps for 1 hour. Recirculation was maintained for 24 hours by releasing clamps and the space was closed via 3/0 suture in reperfusion stage. When the reperfusion process finished, the renal tissues were excised. Dimethyl Sulfoxide (DMSO) group; 0.3 ml, 1% DMSO (Sigma Aldrich Co.) was administered to the rats intraperitoneally (i.p.) on alternate days for 10 days prior to the experiment and latest application was performed 30 minutes before the reperfusion stage. Brusatol group; brusatol (Sigma Aldrich Co.) was administered to animals i.p. at the dose of 0.5 mg/ml every other day for 10 days before the experiment and the administration of last dose was carried out 30 minutes before reperfusion. Brusatol dose was selected based on previous studies (18). Right after, as in RIR group, the IR model was carried out. After all, renal tissue samples were washed and kept frozen.

Biochemical Analysis

Following homogenization, all biochemical analyses were carried out. Malondialdehyde (MDA) level were measured to define lipid peroxidation as defined by Ohkawa et al. (19). SOD activity was gauged with the protocol detected by Sun et al. (20). MPO activity was gauged using a technique improved by Bradley et al. (21). TOS value was determined with an appropriate kit (Rel Assay Diagnostics). TAS value was evaluated with the commercial kit (Rel Assay Diagnostics). OSI, TOS to TAS ratio, was determined as: OSI=[(TOS, μmol H2O2 equivalent/L)/(TAS, mmol Trolox equivalent/L)×10]. TNF-α and IL-1 β levels were determined with the commercially available kit (Elabscience, Wuhan, China).

Statistical Analysis

Statistical analysis was performed by using SPSS 17.0 software. The data were demonstrated as Means±Standard Error Mean (SEM). One way-ANOVA analysis of variance test was applied for all data. Appropriate test was selected for data showing normal distribution. Then Tukey test was used for pairwise comparisons of groups. The differences were accepted significant when $p<0.05$.

RESULTS

MDA levels of kidney tissues raised in RIR and DMSO groups, whereas SOD activity decreased significantly compared to sham group. Contrary to this, these parameters changed significantly due to brusatol treatment (Table I; $p<0.05$). In addition, IL-1β, MPO, TNF-α levels elevated in RIR and DMSO groups, but declined significantly with brusatol treatment (Figure 1; $p<0.05$).

TOS and OSI values were significantly elevated in RIR and DMSO groups compared to sham group and TAS value diminished. However, TAS value raised but TOS and OSI values declined significantly due to brusatol treatment (Figure 2; $p<0.05$).

Table I: The results of MDA (μmol/gr protein) levels and SOD (U/mg protein) activities of all experimental groups.

	MDA (μmol/gr tissue)	SOD (U/mg protein)
SHAM	70.88±2.05[a]	651.75±26.46[a]
RIR	130.91±2.45[a,b]	321.51±6.10[a,b]
DMSO	130.96±2.23[a,b]	318.62±3.08[a,b]
BRUSATOL	75.80±2.03[b]	561.10±8.73[b]

All data was presented as Mean±SEM. a, b: It represents the significant relationship between groups with the same letters. A p-value of <0.05 was considered significant.

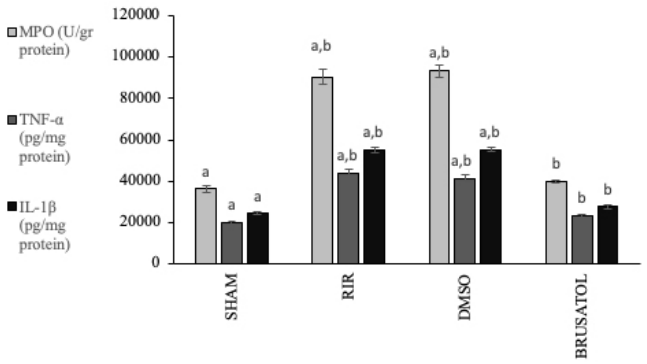

Figure 1: MPO (U/mg protein), TNF-α (pg/mg protein) and IL-1β (pg/mg protein) results and Mean±SEM values of all experimental groups. a,b: It represents the significant relationship between groups with the same letters. A p-value of <0.05 was considered significant.

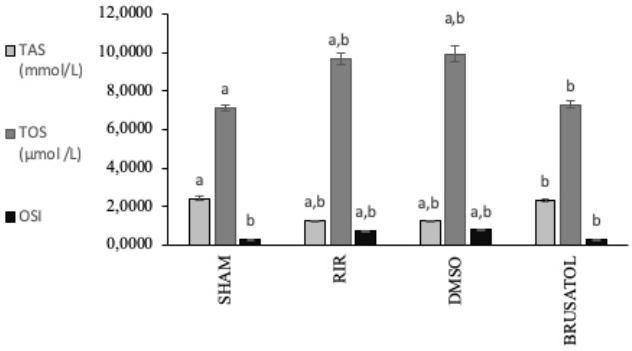

Figure 2: Mean±SEM results of TAS (mmol/L), OSI and TOS (μmol/L) values of all experimental groups. a,b: It represents the significant relationship between groups with the same letters. A p-value of <0.05 was considered significant.

DISCUSSION

AKI is closely associated with high rates of morbidity and mortality. It frequently occurs due to RIR injury (1, 2). Renal ischemia occurs in various medical conditions including organ transplantation, cardiovascular surgery, burn, trauma and shock (22,23). RIR-related necrosis and apoptosis trigger acute renal failure (24). Intracellular alterations such as ROS production, ionic disturbances and cell membrane permeability occur depending on decrease in adenosine triphosphate production during ischemia (25,26). And also, following a hypoxia period, in the reperfusion stage blood flow restarts and oxygen-derived free radicals occur (27). When the oxidant mechanisms overwhelm the antioxidant system, oxidative stress occurs. OSI, TOS to TAS ratio, represents the oxidative stress degree (10). If the inflammatory response impairs renal functions, this leads to progressive chronic kidney disease (28). MPO exists in neutrophils and commonly mentioned as a neutrophil infiltration marker (11). During the kidney ischemia inflammatory factors, ROS and MPO is released due to neutrophil activation and migration. This may exacerbate the renal injury (29). It was found that ROS linked to high activities of MDA reacts with nucleic acids, proteins, and lipids, leads to massive protein oxidation and degradation, and lipid peroxidation in biological membranes (30). IR is associated with lipid peroxidation, which leads to oxidative demolition of the cellular membranes and their catabolites may form harmful metabolites and cell death (10, 11). TNF-α is generated by activated immune cells upon inflammation (31). TNF-α is a protein structured cytokine which is efficient in inflammatory response due to acting on surface adhesion molecules, acute phase protein secretion and endothelial cell permeability (32,33). Ischemic necrosis causes morphologic changes in renal tubular cells (34). Even with progresses in diagnosis and treatment methods, ischemic acute renal failure is a major and common clinical problem (35).

In literature, there are several studies showing the antioxidant and anti-inflammatory properties of brusatol supporting current study. In the present study, reduction of TNF-α, IL-1β levels in RIR model in rats by brusatol, suggests that brusatol decreased IR-induced renal injury. Brusatol has been proven to inhibit amyloid-induced neurotoxicity and decrease ROS (36). Brusatol displayed anti-inflammatory properties and normalized glucose intolerance in mice (37). Brusatol improved the experimental colitis model in rats by decreasing levels of proinflammatory cytokines and increasing the levels of antioxidants (18). Brusatol reduced pro-inflammatory cytokines such as TNF-α and IL-1β against chronic obstructive pulmonary disease-like inflammation in the mouse model (16). In current study, antioxidant and anti-inflammatory properties of brusatol have been shown in RIR model in rats. In RIR group, TAS and SOD decreased while MDA, MPO, TNF-α, IL-1β, TOS, OSI levels were elevated. Brusatol treatment reversed these levels.

Clearly observed in IR studies that inflammation and oxidative stress suppression can provide significant contributions to the treatment of IR. In current study, inflammation and oxidative stress pathways were suppressed by brusatol and it promises hope in the treatment of IR.

CONCLUSIONS

Brusatol provides a protection against IR-induced renal injury. Moreover, further research is necessary for explaining the other protective mechanisms on IR-induced renal tissue damage.

Ethics Committee Approval:

Ethical approval was performed through Atatürk University Experimental Animals Local Ethics Committee (28.03.2019/68). We also declare that this study was conducted in accordance with Research and Publication Ethics.

Conflict of Interest:

The authors have no conflict of interest to declare.

Financial Disclosure:

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