Clinical and Experimental Health Sciences

Original Article / *Özgün Araştırma*

Effect of an Aqueous Garlic Extract on Kidney Damage in an Experimental Model of Sepsis

Sulu Sarımsak Ekstresinin Deneysel Sepsis Modelinde Böbrek Hasarı Üzerine Etkisi

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Abstract

Objective: Sepsis is a systemic inflammatory response against pathogens or substances secreted by pathogens. In this study, the potential protective effect of an aqueous garlic extract (AGE) against sepsis-induced kidney injury.
Methods: Rats were divided into four groups: control, sepsis, sepsis+AGE-garlic, and sepsis+pretreated garlic. Sepsis was induced using cecal ligation and perforation. An AGE was orally administered to rats in the sepsis+pretreated garlic group at a dose of 250 (mg/kg/day) for 15 days prior to sepsis induction. In rats in the sepsis+garlic group, the AGE was administered at a single dose (250 mg/kg) immediately after sepsis induction. Twelve hours after sepsis induction, all rats were decapitated and kidney tissues were taken. Glutathione (GSH) and malondialdehyde (MDA) levels and superoxide dismutase (SOD), tissue factor (TF), catalase (CAT), and myeloperoxidase (MPO) activities were determined in the kidney issue.
Results: Increased MDA levels and MPO activity and decreased GSH level and SOD and CAT activities due to sepsis were reversed by the AGE. TF activity did not change in sepsis. Shortened clot formation time shows increased TF activity. Accordingly, kidney TF activity significantly increased in mice in the pre-treated garlic group.
Conclusion: AGE usage should be considered in developing new sepsis treatment strategies in terms of oxidant and antioxidant balance.
Keywords: Sepsis, kidney, aqueous garlic extract, oxidative stress, tissue factor

Öz

Amaç: Sepsis, patojenlere veya onların salgıladığı maddelere karşı verilen sistemik inflamatuvar bir yanıttır. Bu çalışmada sepsiste oluşan böbrek hasarına karşı sulu sarımsak ekstresinin olası koruyucu etkisi incelenmiştir.
Yöntemler: Çalışmamızda sıçanlar; 'kontrol', 'sepsis', 'sepsis+sarımsak' ve 'sepsis+ön-tedavili sarımsak' grupları olmak üzere 4 gruba ayrılmıştır. Sepsis modeli çekal ligasyon ve perforasyon yöntemi ile oluşturulmuştur. 'Sepsis+ön-tedavili sarımsak' grubuna sepsis oluşumundan 15 gün önce başlanarak, 250 mg/kg/gün dozunda sulu sarımsak ekstresi oral yoldan uygulanmıştır. 'Sepsis+sarımsak' grubunda ise tedavi sepsis uygulamasından hemen sonra tek doz (250 mg/kg) olarak yapılmıştır. Sepsis oluşumundan 12 saat sonra bütün gruplardaki sıçanlar dekapite edilerek, böbrek dokuları alınmıştır. Böbrek dokusunda, glutatyon (GSH) ve malondialdehit (MDA) seviyeleri, süperoksit dismutaz (SOD), doku faktörü (TF), katalaz (CAT) ve miyeloperoksidaz (MPO) aktivitesi tayin edilmiştir.
Bulgular: Sepsise bağlı olarak böbrek dokusunda artan MDA düzeyleri ve MPO aktivitesi ile azalan GSH düzeyleri ve SOD ve CAT aktiviteleri sulu sarımsak ekstresi ile geri çevrilmiştir. TF aktivitesi sepsiste değişmemiştir. Kısalmış pıhtı oluşum zamanı, artmış TF aktivitesini göstermektedir. Bu doğrultuda ön-tedavili sepsis sarımsak grubunda TF aktivitesi artmıştır.
Sonuç: Sulu sarımsak ekstresi kullanımının yeni sepsis tedavi yöntemleri geliştirilirken oksidan-antioksidan dengesi açısından dikkate alınması gerektiğini düşünmekteyiz.
Anahtar Kelimeler: Sepsis, böbrek, sulu sarımsak ekstresi, oksidatif stres, doku faktörü

INTRODUCTION

Sepsis is a condition characterized by a disseminated inflammatory response triggered by a bacterial, viral, or fungal infection. In sepsis, kidney injury frequently occurs as a complication (1). In this condition, kidney function decreases and the ion–water balance becomes impaired (2).

It has been demonstrated that free radicals and the resultant oxidative stress play an important role in sepsis-induced multiorgan damage (3, 4). Proinflammatory markers of oxidative stress include cell and DNA damage, neutrophil recruitment, lipid peroxidation, and protein oxidation (5). Therefore, antioxidant usage may increase survival rates by decreasing oxidative stress in sepsis (6).

Various studies have been conducted to understand the progression of septic kidney damage using animal models of sepsis. The cecal ligation and perforation (CLP) model of polymicrobial peritonitis is frequently used in rats (7). To date, successful treatment strategies have not been developed against sepsis-induced kidney damage. In the present study, the effect of prolonged 15 days AGE usage and

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single-dose aqueous garlic extract (AGE) against sepsis-induced damages in kidney tissue was investigated.

Garlic, *Allium sativum*, has been widely used as a traditional medicine for many years worldwide. It contains 1–3% of sulfur-containing compounds (8). Allicin is the main sulfur-containing compound in garlic (9). It has the ability to penetrate through membranes rapidly, and it can react with thiol-containing proteins and enzymes (10). There are many reports that show the antioxidant activity of garlic and its constituents *in vivo* and *in vitro*, but its role in sepsis-induced kidney damage has not been studied (11-14).

METHODS

Animals

Wistar albino rats (200 to 250 g, either sex) were kept in a room where the temperature was set to 22±2°C and relative humidity to 65–70%. Light cycles were set as 12 h: 12 h light: dark. Standard rat chow was used for feeding. The experimental protocol was approved by the Marmara University Animal Care and Ethics Committee (Protocol Number: 10.2015.mar)

AGE Preparation

Garlic was acquired from Kastamonu, Turkey. It was stored in dry and light protected conditions. It means that standard controlled conditions for plant. In a mortar, peeled garlic (30 g) was crushed with distilled water. It was decanted by pressing, and 60 mL of aqueous garlic was extracted. One milliliter of AGE contained material from 500 mg of garlic (1 mL of AGE extract contains 500 mg garlic, 500 mg/mL) (15, 16). The AGE was stored at 4°C.

Experimental Protocol and Sepsis Induction

Forty rats were equally divided into four groups: control (C), sepsis (S), sepsis+garlic (S+AGE), and sepsis+pretreated garlic (S+pre-AGE). Rats in the C and S groups were supplemented with saline and those in the S+pre-AGE group were supplemented with AGE (250 mg/kg/day orally) for 15 days prior to CLP. In rats in the sepsis+AGE group, a single dose of garlic extract (250 mg/kg/day orally) was given immediately after sepsis induction. In the sham-operated control groups YES, after laparotomy, the cecum was manipulated but left intact (without ligation or perforation). In the S group, rats underwent CLP according to the method described by Fujimura et al. (17).

Minimal dissection was used for midline laparotomy; then, the cecum was ligated just below the ileocecal valve with 3-0 silk ligatures. Using an 18-gauge needle, the cecum was perforated at two locations and, the cecum was gently compressed until the feces were entirely extruded. After feces extrusion, the incision was closed. All rats were resuscitated with saline (3 mL/100 g body weight, subcutaneous.) at the end of the operation. Twenty-four hours after sepsis induction, the rats were decapitated, and kidney tissues were taken. Malondialdehyde (MDA) and glutathione (GSH) levels and superoxide dismutase (SOD), myeloperoxidase (MPO), catalase (CAT), and tissue factor (TF) activities were determined in kidney tissue homogenates by the methods by Yagi (18), Beutler (19), Mylorie et al. (20), Hillegas et al. (21), Aebi (22), and Ingram (23) respectively.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA), and all data were expressed as mean±standard deviation. Data groups were compared with analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. A p value of less than 0.05 was considered significant.

RESULTS

MDA and GSH Levels

Kidney MDA levels significantly increased in the S group in comparison with those in C group (Table 1). The increase seen in the S group was in the S+pre-AGE group (Table 1). However, a single dose of AGE administration did not significantly change the MDA level in the S group.

Kidney GSH levels significantly decreased in the S group in comparison with those in the control group. Pre-AGE administration significantly increased GSH levels in the S group (Table 1).

Kidney SOD, CAT, and MPO Activities

Kidney SOD activity decreased in the S group in comparison to that in the control group (Figure 1). A single dose of AGE administration significantly increased SOD activity in the S group (Figure 1).

Kidney CAT activity decreased in the S group but, this decrease was not statistically significant (Figure 2). Both AGE administrations significantly increased CAT activity in S group (Figure 2). Kidney MPO activity insignificantly increased in the S group (Figure 3). Both AGE administrations significantly decreased MPO activity in the S group (Figure 3).

Kidney TF Activity

Tissue factor activity was expressed in seconds. Shortened clot formation time shows increased TF activity. TF activity did not significantly change in the S group, but in S+pre-AGE group, TF activity significantly increased (Table 2). A single dose of AGE administration did not significantly change TF activity in the S group (Table 2).

Table 1. Malondialdehyde and glutathione levels in all groups

	C (n=8)	S (n=8)	S+pre-AGE (n=8)	S+AGE (n=8)
MDA (nmol MDA/g tissue)	63.41±3.32	112.3±8.,75 $^{+++}$, $^{++}$	67.92±8.04	95.65±3.83
GSH (µg GSH/g tissue)	138.0±8.30	105.3±3.82 $^{+++}$, $^{+}$	129.70±2.57	126.2±4.54

Values are given as mean±standard deviation (SD)

C: control; S: sepsis; S+pre-AGE: AGE administration 15 days prior to sepsis induction; S+AGE: single dose of AGE administration after sepsis induction; (èèè): p<0.001 versus control group; (+): p<0.05 versus S+pre-AGE group

(++): p<0.01 versus S+pre-AGE group

Table 2. Kidney tissue factor activities in all groups

	C (n=6)	S (n=8)	S+pre-AGE (n=8)	S+AGE (n=8)
TF activity (sec.)	44.67±1.51	42.83±1.60 +	39.75±2.49	44.20±3.03

Values are given as mean±standard deviation (SD)

C: Control; S: sepsis; S+pre-AGE: AGE administration 15 days prior to sepsis induction; S+AGE: single dose of AGE administration after sepsis induction; TFa: tissue factor activity; sec.: seconds (+): p<0.05 versus the S+pre-AGE group

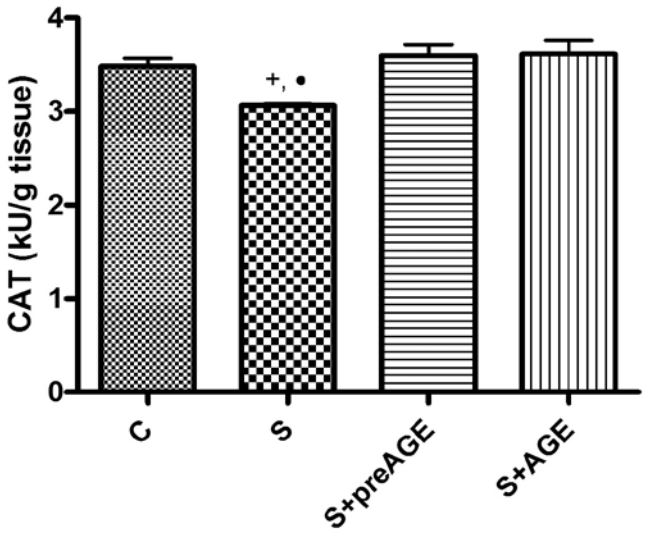

Figure 1. Superoxide dismutase activities in all groups
C: control; S: Sepsis; S+pre-AGE: AGE administration 15 days prior to sepsis induction; S+AGE: Single dose of AGE administration after sepsis induction; (ééé): p<0.001 versus the control group, (•): p<0.05 versus the S+AGE group

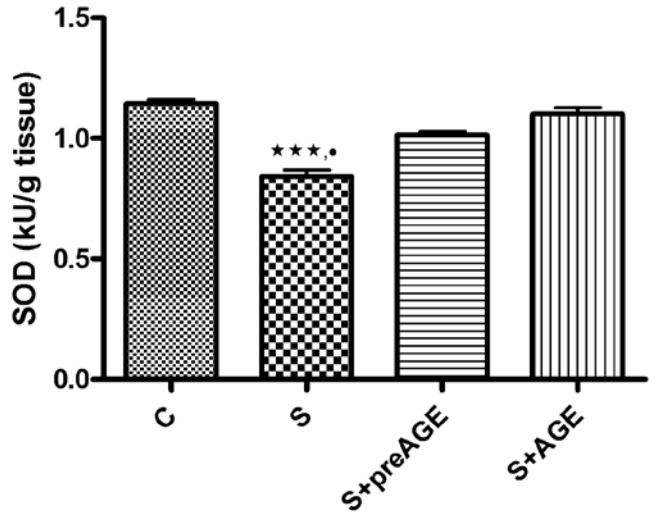

Figure 2. Catalase activities in all groups
C: Control; S: Sepsis; S+pre-AGE: AGE administration 15 days prior to sepsis induction; S+AGE: single dose of AGE administration after sepsis induction; (+): p<0.05 versus the S+pre-AGE group; (•): p<0.05 versus S+AGE group

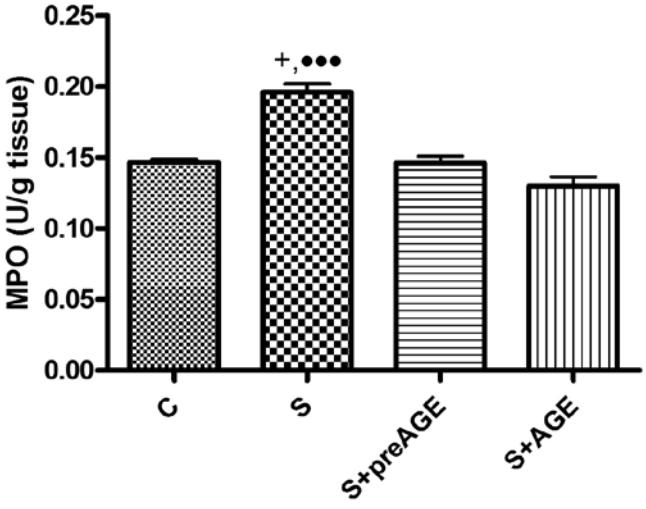

Figure 3. Myeloperoxidase activities in all groups
C: Control; S: Sepsis, S+pre-AGE: AGE administration 15 days prior to sepsis induction; S+AGE: Single dose of AGE administration after sepsis induction; (+): p<0.05 versus S+pre-AGE group; (•••): p<0.0001 versus the S+AGE group

DISCUSSION

Garlic-derived allium derivatives have been shown to exert antibiotic, anticancer, antithrombotic, and lipid-lowering cardiovascular effects (24). In the present study, renal pathologic changes induced by oxidative damage due to experimental sepsis and the putative protective roles of AGE against this damage were investigated. In kidney tissue, AGE administration alleviated the sepsis-induced oxidative damage by significantly decreasing the MDA level and MPO activity and increasing the GSH level and SOD and CAT activities. Furthermore, TF activity, which did not change in sepsis, increased by AGE administration that was given 15 days prior to sepsis induction. Sepsis-associated oxidative damage in kidney tissue was prevented by the antioxidant properties of garlic.

Sepsis is an inflammatory response that affects various organs and systems; it also contributes to an inflammatory response, microvascular hypoperfusion, organ dysfunction, and increased mortality. In sepsis, the kidney is subjected to inflammatory cytokines and ischemia. Sepsis also causes widespread tubular cell apoptosis (25).

In sepsis, an increase in the production of ROS leads to multiorgan dysfunction, mostly in the kidneys, lungs, liver, heart, and intestines. These dysfunctions are known to result from bacterial toxins and enzymes, the effects of mediators, impaired perfusion, and disseminated intravascular coagulation (26).

In the present study, the MDA level was significantly increased in kidney tissue. AGE administration prior to sepsis induction inhibited MDA elevations in kidney tissue, but single dose of AGE administration did not significantly change the increased kidney MDA level. Restored control levels might be related to the maintained cellular integrity that was achieved by AGE administration.

As an enzymatic antioxidant, SOD is particularly important for the intracellular destruction of phagocytized bacteria and granulocyte function (27). GSH, which is also a non-enzymatic antioxidant, protects tissues against oxidative stress (28). In our study, kidney tissue GSH level and SOD and CAT activities decreased in the S group in comparison to those in the control group. Pre-AGE administration increased GSH level and CAT activity, while a single dose of AGE administration was effective in increasing only SOD activity.

The heme enzyme MPO, found in neutrophils, uses a superoxide anion to produce hypochlorous acid, which is the major oxidant for its immune function (29). Thus, tissue-associated MPO activity is considered to correlate to the degree of inflammatory damage. In the present study, the increase in kidney MPO activity in the S group compared to that in the C group might be related to neutrophil accumulation in sepsis-induced oxidative injury. However, the decrease in kidney MPO activity in both the S+AGE and S+pre-AGE groups might be considered to explain the anti-inflammatory effect of AGE.

Due to its receptor activity for factor VII, TF is the primary initiator of the blood coagulation cascade, while also ensuring rapid hemostasis in the case of organ damage (30). TF activity did not significantly change in the S group. As a shortened clot formation time shows increased TF activity, AGE administration prior to sepsis induction significantly increased kidney TF activity. Increase in kidney TF activity with pre-treated AGE administration may help eliminate the sepsis-induced risk of bleeding in kidney tissues.

CONCLUSION

In conclusion, long-term AGE administration has great potential for preventing the oxidation and inflammation of the kidneys seen after sepsis induction. The mechanism of this effect might be the maintenance of cellular integrity by the AGE during sepsis.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee on Research Animals of Marmara University (10.2015.mar).

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