Sesamol attenuates systemic inflammation-associated acute kidney injury in polymicrobial infectious rats

Y. H. Li¹; D. Z. Hsu¹ and M. Y. Liu¹,²

¹Department of Environmental and Occupational Health, National Cheng Kung University College of Medicine, 138 Sheng-Li Road, Tainan 70428, Taiwan
²Research Center for Environmental and Occupational Health and Preventive Medicine, National Cheng Kung University College of Medicine, 138 Sheng-Li Road, Tainan 70428, Taiwan

Bacterial infection is associated with systemic inflammatory response, which is involved in the pathogenesis and development of acute kidney injury (AKI). Sesamol protects against endotoxin-induced inflammation and organ failure; however, the effect of sesamol on systemic inflammation-initiated AKI in polymicrobial infectious rats is unclear. Therefore, we investigated the effect of sesamol on systemic inflammation-associated AKI in cecal ligation and puncture (CLP)-treated rats. Rats were given two subcutaneous doses of sesamol (10 mg/kg) 0 and 6 h after CLP. Serum and kidney tissue were sampled 12 h after CLP. Proinflammatory mediators, such as interleukin (IL)-1β, IL-6, and nitrite production were detected. Renal dysfunction indicators, including blood urea nitrogen (BUN), creatinine (CRE), and neutrophil gelatinase-associated lipocalin (NGAL) levels were examined. In addition, neutrophil infiltration was also assessed by myeloperoxidase (MPO) activity. The levels of IL-1β and IL-6, nitrite production, BUN, CRE, NGAL, and MPO activity were significantly higher in rats after CLP. Sesamol significantly inhibited IL-1β, IL-6, nitrite production, BUN, CRE, NGAL, and MPO activity in CLP-treated rats. In summary, sesamol attenuated AKI by inhibiting neutrophil-initiated systemic inflammation in polymicrobial infectious rats.

Keywords: sesamol; systemic inflammation; acute kidney injury; cecal ligation and puncture; polymicrobial infection

1. Introduction

Infection is common in the critically ill and often occurs because of the severity of the patient's illness [1]. One study reports that 51% of intensive care unit (ICU) patients are infected, and 71% receive antimicrobial therapy [2]. Gram-positive bacteria and Gram-negative bacteria are the major causative organisms in infected patients. The mortality rate in infected patients is more than twice that in uninfected patients.

Bacterial infection is closely associated with systemic inflammatory response [3]. Overwhelming systemic inflammation is a risk factor in the pathogenesis of acute organ damage [4]. For example, acute kidney injury (AKI) is strongly associated with systemic inflammation [5, 6]. Neutrophils are the major leukocytes promptly recruited to the inflamed loci in response to infection or tissue injury [7]. Interleukin (IL)-1β, IL-6, and nitric oxide (NO) are the neutrophil-derived proinflammatory mediators involved in systemic inflammation [8, 9] and are important components of both the initiation and extension of inflammation in AKI [10]. In addition, neutrophil gelatinase-associated lipocalin (NGAL) is highly expressed in the damaged kidney in response to inflammatory signals [11]. NGAL is commonly used as an early and predictive biomarker for AKI.

Sesamol, the major constituent of sesame oil, is resistant to oxidative deterioration [12]. Sesamol is potent anti-oxidative [13] and anti-inflammatory [14], and it protects against multi-organ failure [15]. However, the effect of sesamol on systemic inflammation-associated AKI induced by bacterial infection remains unknown. In addition, cecal ligation and puncture (CLP), a polymicrobial infection animal model, is widely used for inducing a systemic inflammatory response [16]. We investigated the effect of sesamol on systemic inflammation-initiated AKI in CLP-treated rats.

2. Materials and methods

2.1 Chemicals

Sesamol was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Animals

Male SPF Wistar rats, weighing 200-300 g, were obtained from and housed in our institution’s Laboratory Animal Center. Rats were housed individually in a room with a 12-h light/dark cycle and central air conditioning (25°C, 70% humidity). Rats were allowed free access to tap water and were fed a rodent diet (Richmond Standard, PMI Feeds, Inc.;
St. Louis, MO, USA). The animal care and experimental protocols were in accordance with nationally approved guidelines.

2.3 Experimental design

**Experiment I.** Rats were divided into two groups of five. Sham group: rats were given a sham operation without CLP; and CLP group: rats were given CLP only. Serum IL-1β, IL-6, nitrite, blood urea nitrogen (BUN), and creatinine (CRE) levels were determined 0, 1, 3, and 12 h after CLP. Renal NGAL level was also determined 12 h after CLP.

**Experiment II.** Rats were divided into five groups of five. Group I: rats were given a sham operation without CLP. Group II: rats were given CLP only. Groups III-V: rats were given subcutaneous (s.c.) sesamol (1, 3, and 10 mg/kg, respectively) 0 and 6 h after CLP. Serum IL-1β, IL-6, and nitrite levels were determined 12 h after CLP.

**Experiment III.** Rats were divided into four groups of five. Group I: rats were given a sham operation without CLP. Group II: rats were given sesamol (10 mg/kg, s.c.) at 0 and 6 h without CLP. Group III: rats were given CLP only. Group IV: rats were given sesamol (10 mg/kg, s.c.) 0 and 6 h after CLP. Renal IL-1β and IL-6, and nitrite production were determined 12 h after CLP. Renal dysfunction was evaluated by BUN, CRE, and NGAL levels. In addition, renal myeloperoxidase (MPO) activity was examined 12 h after CLP.

### 2.3.1 CLP

Rats were anesthetized using light diethylether and then shaved over the anterior abdominal wall. A 2-cm-long midline incision, sufficient to expose the cecum and the adjacent intestine, was made. The ligated cecum was punctured twice with an 18-gauge needle, after which the cecum was gently squeezed to exude fecal matter. The abdominal incision was then closed, and 1 mL of saline was injected (s.c.) for fluid resuscitation [17].

### 2.3.2 Blood collection

Rat blood samples were collected from the femoral vein under light ethylether anesthesia. Blood was drawn via venipuncture into serum separation tubes, allowed to clot for 30 min at room temperature, and then centrifuged at 1000 × g for 10 min at 4°C.

### 2.3.3 Measuring IL-1β and IL-6 levels in serum and kidney tissue

Kidney tissue was homogenized in deionized water (1:10; wt/vol) and centrifuged at 2500 × g for 30 min at 4°C. The IL-1β and IL-6 levels in serum and in kidney tissue supernatant were measured quantitatively using enzyme-linked immunoassorbent assay (ELISA) kits (DuoSet; R&D Systems Inc., Minneapolis, MN, USA). Briefly, a 96-well immunoassay plate was coated with capture-antibody (100 µL/well) overnight at room temperature, followed by a blocking step. Recombinant cytokines ranging from 62.5 to 4000 pg/mL were used as standards. One hundred microliters of test sample and serial standards diluted in sample buffer (PBS containing 1% [w/v] BSA [pH 7.6]) were incubated at room temperature for 2 h. After serum samples or supernatants had been incubated with 100 µL of biotinylated rabbit antibody (anti-rat IL-1β or anti-rat IL-6), streptavidin-conjugated horseradish peroxidase was added for an additional 20-min incubation at room temperature. The peroxidase reaction was initiated by adding 100 µL of 3',3',5',5'-tetramethylbenzidine/H2O2 (R&D Systems) and then, after 30 min, stopped by adding 50 µL of 0.5 M H2SO4. The absorbance was measured at 450 nm with an ELISA reader [18].

### 2.3.4 Measuring nitrite production in serum and kidney tissue

Kidney tissue was homogenized in deionized water (1:10; wt/vol) and centrifuged at 2500 × g for 30 min at 4°C. The amounts of nitrite in serum and in kidney tissue supernatant were measured after the Griess reaction by incubating 100 µL of sample with 100 µL of Griess reagent (Sigma-Aldrich) at room temperature for 20 min. The absorbance was measured at 550 nm using a spectrophotometer [19]. Nitrite concentration was calculated by comparing it with a standard solution of known sodium nitrite concentrations.

### 2.3.5 Assessing renal dysfunction

Renal dysfunction was assessed by measuring rises in serum levels of BUN and CRE. Serum samples were spotted to slides and evaluated for BUN and CRE using a blood biochemical analyzer (DRI-CHEM 3500 s; Fujifilm, Kanagawa, Japan).
2.3.6 Measuring NGAL level in kidney

Kidney tissue was homogenized in 1X phosphate buffer saline (PBS) (1:10; wt/vol) and centrifuged at 2500 × g for 30 min at 4°C. Renal NGAL was quantitatively measured using ELISA kits (BioVendor Laboratorni Medicina a.s.; Modrice, Czech Republic). Briefly, a 96-well immunoassay plate was precoated with anti-rat NGAL antibody, followed by a blocking step. Recombinant rat NGAL ranging from 156 to 5000 pg/mL was used as the standard. One hundred microliters of test sample and serial standards diluted in sample diluent buffer were incubated at room temperature for 1.5 h. After the supernatants had been incubated with 100 µL of biotinylated anti-rat NGAL antibody at room temperature for 1 h, avidin-biotin-peroxidase complex working solution was added and the supernatants were incubated for an additional 30 min at room temperature. The peroxidase reaction was initiated by adding 90 µL of 3',3',5',5'-tetramethylbenzidine/H2O2 for 30 min and then stopped by adding 100 µL of 0.5 M H2SO4. The absorbance was measured at 450 nm with an ELISA reader.

2.3.7 Measuring MPO activity in kidney tissue

Kidney tissue was homogenized in 20 mM of phosphate buffer (pH 7.4) and then centrifuged (15,400 × g for 10 min at 4°C). The pellet was resuspended in 1 mL of 50 mM phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide. The suspension was subjected to four cycles of freezing and thawing, and then centrifuged (15,400 × g for 5 min at 4°C). Supernatant (0.5 ml) was mixed with tetramethylbenzidine (0.5 ml) and incubated for exactly 1 min. The reaction was stopped by adding 0.5 ml of 2 N H2SO4. The spectrophotometer was then used to measure the absorbance at 405 nm. MPO activity is shown as the absorbance at 405 nm/min/mg protein [20].

2.4 Protein assay

Protein concentration in kidney tissue was determined using protein assay dye (Bio-Rad Laboratories, Hercules, CA, USA), and bovine serum albumin (BSA) was used as a standard.

2.5 Statistical analysis

Data are expressed as means ± standard deviation (SD). Differences between groups were analyzed using a Mann-Whitney U test. Significance was set at \( P < 0.05 \).

3. Results

3.1 The time course of the release of proinflammatory mediators and of BUN, CRE, and NGAL levels in CLP-treated rats

We examined the time course of CLP on the circulating proinflammatory mediators released and on renal dysfunction. Twelve hours after CLP, serum IL-1β and IL-6, and nitrite levels were significantly higher in the CLP group than in the Sham group (A-C) (Fig. 1). To further understand the development of AKI after CLP, serum BUN and CRE, and renal NGAL levels were also examined. Twelve hours after CLP, serum BUN (D) and CRE (E), and renal NGAL (F) levels were also significantly higher in the CLP group than in the Sham group.
3.2 Dose-response of sesamol on the release of proinflammatory mediators in CLP-treated rats

We examined the dose-response of sesamol on the release of circulating proinflammatory mediators. Serum IL-1β, IL-6, and nitrite levels were detected 12 h after CLP. Serum IL-1β (A), IL-6 (B), and nitrite (C) levels were significantly higher in the CLP group (Group II) than in the Sham group (Group I) (Fig. 2). Sesamol at the dose of 10 mg/kg (Group V) significantly reduced IL-1β, IL-6, and NO levels compared with the CLP group.

Fig. 2 Dose-response of sesamol on the release of systemic proinflammatory mediators in CLP-treated rats. Rats were divided into five groups of five. Group I: rats were given a sham operation without CLP. Group II: rats were given CLP only. Groups III-V: rats were given sesamol (SM) (1, 3, and 10 mg/kg, respectively, s.c.) 0 and 6 h after CLP. Serum IL-1β (A), IL-6 (B), and nitrite (C) levels were determined 12 h after CLP. ND: not detected. Data are means ± SD (n = 5). * P < 0.05 compared with Group I. † P < 0.05 compared with Group II.
3.3 Sesamol inhibited the release of renal proinflammatory mediators in CLP-treated rats

We examined the effects of sesamol on the release of renal proinflammatory mediators. Renal IL-1β (A), IL-6 (B), and nitrite (C) levels were significantly higher in the CLP group (Group III) than in the Sham group (Group I) and the Sesamol-alone group (Group II) (Fig. 3). However, sesamol (Group IV) significantly inhibited IL-1β, IL-6, and nitrite levels after CLP compared with the CLP group.

Fig. 3 The effect of sesamol on the release of renal proinflammatory mediators in CLP-treated rats. Rats were divided into four groups of five. Group I: rats were given a sham operation without CLP. Group II: rats were given sesamol (SM) (10 mg/kg, s.c.) at 0 and 6 h without CLP. Group III: rats were given CLP only. Group IV: rats were given SM (10 mg/kg, s.c.) 0 and 6 h after CLP. Renal IL-1β (A), IL-6 (B), and nitrite (C) levels were determined 12 h after CLP. Data are means ± SD (n = 5). * P < 0.05 compared with Groups I and II. † P < 0.05 compared with Group III.

3.4 Sesamol inhibited BUN, CRE, and NGAL levels in CLP-treated rats

We examined the effect of sesamol on the development of AKI in CLP-treated rats. Serum BUN (A) and CRE (B), and renal NGAL (C) levels were significantly increased in the CLP group (Group III) compared with the Sham group (Group I) and the Sesamol-alone group (Group II) (Fig. 4). However, sesamol (Group IV) decreased serum BUN and CRE, and renal NGAL levels after CLP compared with the CLP group.

Fig. 4 The effect of sesamol on BUN, CRE, and NGAL levels in CLP-treated rats. Rats were divided into four groups of five. Group I: rats were given a sham operation without CLP. Group II: rats were given sesamol (SM) (10 mg/kg, s.c.) at 0 and 6 h without CLP. Group III: rats were given CLP only. Group IV: rats were given SM (10 mg/kg, s.c.) 0 and 6 h after CLP. Serum BUN (A) and CRE (B), and renal NGAL (C) levels were determined 12 h after CLP. Data are means ± SD (n = 5). * P < 0.05 compared with Groups I and II. † P < 0.05 compared with Group III.
3.5 Sesamol inhibited renal MPO activity in CLP-treated rats

We examined the effect of sesamol on MPO activity, a marker of neutrophil infiltration. Renal MPO activity was significantly increased in the CLP group (Group III) compared with the Sham group (Group I) and the Sesamol-alone group (Group II) (Fig. 5). However, sesamol (Group IV) significantly decreased MPO activity after CLP compared with CLP group.

![Fig. 5 The effect of sesamol on renal MPO activity in CLP-treated rats. Rats were divided into four groups of five. Group I: rats were given a sham operation without CLP. Group II: rats were given sesamol (SM) (10 mg/kg, s.c.) at 0 and 6 h without CLP. Group III: rats were given CLP only. Group IV: rats were given SM (10 mg/kg, s.c.) 0 and 6 h after CLP. Renal MPO activity was determined 12 h after CLP. Data are means ± SD (n = 5). * P < 0.05 compared with Groups I and II. † P < 0.05 compared with Group III.]

4. Discussion

In present study, we showed that sesamol protected against AKI, that it decreased proinflammatory mediator levels, and that it reduced neutrophil infiltration. We hypothesize that inhibiting neutrophil infiltration is involved in the protective effect of sesamol on systemic inflammation-associated AKI in polymicrobial infectious rats.

Sesamol protected against AKI by inhibiting the release of polymicrobial infection-induced proinflammatory mediators. CLP is a well-known murine model that causes polymicrobial infection with systemic inflammation. Studies indicate that polymicrobial infection triggers the release of proinflammatory mediators in the circulation [21] and cause AKI [22]. However, they provide no evidence of a correlation between systemic inflammation and AKI after a polymicrobial infection. The upregulation of inflammatory mediators accelerates systemic inflammation and tissue injury [23, 24]. The inhibition of IL-1β and IL-6 levels [25], and lower levels of inducible nitric oxide synthase-derived NO production [26] protect against AKI in endotoxemic mice. Moreover, NGAL is highly expressed in patients with septic AKI [27] and other types of AKI [28, 29]. Sesamol inhibited the release of proinflammatory mediators and reduced BUN, CRE, and NGAL levels. We hypothesize that sesamol attenuates AKI by inhibiting systemic inflammation after a polymicrobial infection in rats.

Inhibiting neutrophil infiltration may be how sesamol protects against systemic inflammation-induced AKI. A large infiltration of neutrophils is involved in the pathogenesis of AKI [30]. Reducing renal neutrophil infiltration or inhibiting neutrophil-derived inflammatory mediators may attenuate AKI [31] or diminish the acute inflammatory response [32]. Sesamol significantly inhibited neutrophil infiltration. This may be at least partially involved in sesamol’s protection against systemic inflammation-associated AKI.

Sesamol may have the potential for preventing AKI in patients with systemic inflammation. Sesamol has potent anti-inflammatory properties [33, 34], protects against organ damage [35-37], and has no adverse effects when used in appropriate doses. Inhibiting neutrophil-initiated proinflammatory mediators may be a potently therapeutic approach for preventing AKI in patients. However, more investigation is needed to confirm this. In summary, we hypothesize that sesamol attenuates AKI by inhibiting neutrophil-initiated systemic inflammation in polymicrobial infectious rats.

Acknowledgements This study was supported by grants 98-2312-B-006-002-MY3 (to D. Z. Hsu) and 96-2628-B-006-038-MY3 (to M. Y. Liu) from the National Science Council, Taiwan.

References


