Protective effect of *Aloe vera* on polymicrobial sepsis in mice

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**Article info**

**Abstract**

Sepsis is an acute life-threatening clinical condition and remains the major cause of death in intensive care units. The primary pathophysiologic event central to the septic response is an overwhelming activation of the inflammatory system and countervailing response from the anti-inflammatory system. However, the cause of this perturbation has yet to be elucidated. In this study, we report that *Aloe vera* therapeutically reverses the lethality induced by cecal ligation and puncture (CLP), a clinically relevant model of sepsis. The administration of *Aloe vera* ameliorated the multiple organ dysfunction syndrome, as evidenced by the serum levels of biochemical parameters and histological changes. In order to investigate the pharmacological mechanism of *Aloe vera*, the levels of the cytokines, tumor necrosis factor (TNF-α), interleukin (IL)-1β, and IL-6 were determined by ELISA at various time points. The increases in the levels of TNF-α, IL-1β, and IL-6 were attenuated by *Aloe vera*. In vivo administration of *Aloe vera* also markedly enhanced bacterial clearance. Our findings suggest that *Aloe vera* could be a potential therapeutic agent for the clinical treatment of sepsis.

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1. Introduction

Sepsis, leading to multiple organ failure, including renal and hepatic failure, remains a leading cause of mortality and morbidity in intensive care units, affecting more than 500,000 persons per year in the United States (Scott et al., 2002; Hotchkiss and Karl, 2003). An uncontrolled hyperinflammatory response and inappropriate cytokine response during early sepsis is proposed to be the main cause of multiple organ dysfunction syndrome (MODS) during early sepsis. Controlling inflammation during early sepsis may therefore reduce organ injury and prevent death after septic insult.

Aloes have been used therapeutically, certainly since Roman times and perhaps since long before (Crosswhite and Crosswhite, 1984), and different properties have been ascribed to the inner, colorless, leaf gel and to the exudates from the outer layers. Previous studies have suggested wound-healing, antibiotic, and ant carcinogenic properties of the active compounds present in the leaves of *Aloe vera* (Davis et al., 1994; Chen et al., 2004, 2007). The various irritant-induced edema models have indicated a broad spectrum of anti-inflammatory activity for *Aloe vera* (Davis et al., 1989) and other groups have reported reduced leukocyte adherence and TNF-α levels, elevated IL-10 levels, and enhanced healing of gastric ulcer (Eamlamnam et al., 2006). Recently, *Aloe vera* was reported to modulate Salmonella OmpR-mediated inflammation (Rishi et al., 2008).

Therefore, in this study, we hypothesized that *Aloe vera* may reduce inflammatory processes, organ injury, and death during early sepsis induced by cecal ligation and puncture (CLP). We have utilized the CLP model, which closely resembles the human sepsis syndrome with respect to the hyperactive inflammatory process, cytokine generation, and development of fulminant multiple organ failure.

2. Materials and methods

2.1. Animals and sepsis model

All animal protocols were approved by the Animal Care Committee of Sungkyunkwan University. We used ICR male mice (Dae Han Biolink Co., LTD, Eum-sung, Korea), and CLP was performed as described previously by Wichterman et al. (1980). Briefly, animals (27–29 g) were anesthetized with an intramuscular injection of ketamine (75 mg/kg, Yuhon Corporation, Seoul, Korea) and xylazine (20 mg/kg, Bayer, Germany). After a 10-mm midline incision, the cecum was care fully exposed to avoid damage to the blood vessels. The cecum was then ligated just distal to the ileocecal valve without causing intestinal obstruction, and the cecal stump was punctured twice with a 20-gauge needle. A small amount of stool was extruded to ensure patency of the puncture sites. The cecum was then extruded back into its normal intraabdominal position, and the abdomen was closed in two layers. All animals received normal saline (20 mL/kg) subcutaneously immediately after surgery (i.e. fluid resuscitation). Sham-operated animals were subjected to laparotomy and intestinal manipulation; however, the cecum was neither ligated nor punctured.
2.2. Administration of Aloe vera

The lyophilized cellulase-treated Aloe gel used in this study was provided by Univera, Incorporation. The basic processing methodology of the Aloe vera gel, which involves incubation of Aloe gel with cellulose, termination of the reaction by heating, and then passage through a charcoal column to remove anthraquinones and other colored substances, was described in detail in an earlier report (Qui et al., 2000). The lyophilized Aloe vera gel was dissolved in phosphate-buffered saline (vehicle, pH 7.4) and administered intravenously immediately after CLP operation. The dose of Aloe vera was selected based on previous reports (Akev et al., 2007; Wang et al., 2001). The animals were randomly assigned to the following four groups: (a) vehicle-treated sham, (b) Aloe-treated sham, (c) vehicle-treated CLP, (d) Aloe-treated CLP. Because no differences between vehicle-treated and Aloe-treated mice in the sham groups were found in any parameters, groups (a) and (b) were pooled to simplify presentation and are referred to as sham.

2.3. Survival studies

To determine the effect of Aloe vera (25 and 50 mg/kg) on mortality from CLP-induced sepsis, survival studies were performed. All mice had free access to water and food and were frequently monitored by dedicated research personnel to determine 10-day survival statistics.
2.4. Assessment of heart, renal and hepatic function after sepsis

Under anesthesia, blood samples were collected from the abdominal inferior vena cava 1, 3, 6, 12, 24, and 48 h after CLP and treatment with vehicle or Aloe vera (50 mg/kg). The serum was separated by centrifugation at 10,000 \( \times \) g for 5 min at 4 \(^\circ\)C. Serum lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, and alanine aminotransferase (ALT) levels were determined using Hitachi 7600 automatic analyzer (Hitachi, Tokyo, Japan).

2.5. Histological Analysis

Twenty-four hours after CLP, tissue samples of heart, kidney, liver, and lung were removed for histological analysis. Each sample was fixed by immersion in 10% neutral-buffered formalin. The sample was then embedded in paraffin, sliced into 5-\( \mu \)m sections, and stained with hematoxylin-eosin for a blinded histological assessment. The degree of congestion, inflammatory cell infiltration, necrosis, and degeneration were evaluated semiquantitatively according to the method reported by Frei et al. (1984). The histological changes were evaluated in random, non-consecutive, \( \times 200 \) histological fields (Olympus BX51/Olympus DP71, Olympus, Japan).

2.6. Measurement of systemic cytokines by enzyme-linked immunoassay

The concentrations of TNF-\( \alpha \), IL-6, and IL-1\( \beta \) were determined using enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences, San Diego, CA) according to the manufacturer’s instructions. For measurement of CLP-induced serum cytokines, blood samples were collected 1, 3, 6, 12, 24, and 48 h after CLP and treatment with vehicle or Aloe vera (50 mg/kg).
2.7. Measurement of blood and peritoneal bacterial load

Twenty-four hours after CLP and treatment with vehicle or Aloe vera (50 mg/kg), mice were anesthetized and peritoneal fluid was collected. After serial dilutions with PBS, peritoneal fluid was cultured overnight on blood-agar base plates (Trypticase Soy Agar Deeps, Becton Dickinson, USA) at 37°C, and colony-forming units (CFUs) were counted.

2.8. Statistical Analysis

Survival data were analyzed by the Kaplan-Meier curve and log-rank test. All other data were analyzed by two-way analysis of variance (ANOVA), and the Bonferroni test was used for post hoc comparisons. The differences between the groups were considered statistically significant at P value < 0.05. The results are presented as mean ± S.E.M.

3. Results

3.1. Effect of Aloe vera on CLP-induced lethality

CLP-induced sepsis showed 70% survival rate on the first day of observation and reached a stable 15% survival rate on the sixth day. Log-rank analysis of the 10-day survival curves demonstrated that Aloe vera at doses of 25 and 50 mg/kg provided a significant level of protection. 25 mg/kg Aloe vera showed 95% survival rate on the first day of observation and reached a plateau on the sixth day with a survival rate of 40% (P=0.0203). Furthermore, 50 mg/kg Aloe vera showed 95% survival rate on the first day and reached a plateau on the sixth day with a survival rate of 55% (P=0.0026) (Fig. 1).

3.2. Effect of Aloe vera on organ injuries induced by CLP

The serum level of LDH was significantly increased at 1 h after CLP and was maximal near 12 h (2251.3 ± 178.3 U/L) and sustained until 24 h after CLP (1953.5 ± 228.1 U/L). Treatment with 50 mg/kg Aloe vera markedly attenuated the increases at 12 and 24 h after CLP (Fig. 2). In the CLP groups, the serum level of BUN was not significantly higher than those of sham groups at all time points and was maximal near 12 h after CLP (0.7 ± 0.1 mg/
Although treatment with Aloe vera at a dose of 25 mg/kg significantly suppressed the increase at 6 h, no differences were observed at the other time points. However, Aloe vera treatment at a dose of 50 mg/kg significantly attenuated the increases induced by CLP at 3, 6, 12, and 24 h after CLP (Fig. 4). The serum level of ALT was significantly increased after CLP compared with that after sham operation. ALT level peaked at 180.7 ± 8.0 U/L at 24 h after CLP and treatment with Aloe vera at a dose of 50 mg/kg significantly attenuated this increase (Fig. 5).

3.3. Histological Analysis

The histological features reveal normal cell structure in the heart, kidney, liver and lung of sham-operated animals (data not shown). CLP induced marked histopathological changes (congestion, inflammatory cell infiltration, necrosis, and degeneration) in tissue sections from septic animals. These pathological changes were inhibited by treatment with 50 mg/kg Aloe vera (Fig. 6).

3.4. Effect of Aloe vera on Cytokine Levels

TNF-α, IL-1β, and IL-6 concentrations were very low in sham surgery control mice. In contrast, serum TNF-α and IL-1β concentrations in mice undergoing 20-gauge CLP were increased at 1 h and were maximal at 3 h. The increase in TNF-α concentration at 3 and 12 h after CLP was attenuated by Aloe vera (50 mg/kg) treatment (Fig. 7), whereas the level of IL-1β at 1, 3, 6, and 12 h after CLP was suppressed by Aloe vera (50 mg/kg) treatment (Fig. 8). The in-
creased serum level of IL-6 at 6 and 12 h after CLP was significantly prevented by 50 mg/kg Aloe vera treatment (Fig. 9).

3.5. Bacterial counts in peritoneal fluid after CLP sepsis

The effect of Aloe vera on bacterial clearance was determined by counting CFUs. 24 h after CLP operation, the intraperitoneal bacterial counts were significantly increased compared with that in sham groups. In treated mice, Aloe vera significantly decreased the CFUs in peritoneal fluid by 75.5%, 24 h after CLP (Fig. 10).

4. Discussion

Despite improvements in the management of septic patients through systemic antibiotics, aggressive surgical intervention, and careful monitoring, septic shock and multiple organ failure continue to be the most common cause of death in surgical care units (Jones et al., 2008). Xigris® (drotrecogin alfa (activated), Eli Lilly), a recombinant form of human activated protein with antithrombotic, anti-inflammatory and profibrinolytic properties, is the first and only FDA-approved therapy for severe sepsis in January 2002 (Esmon, 1992). However, the clinical use of Xigris® is still controversial due to concerns on the increased risk of serious bleeding in patients treated with Xigris® (Abraham et al., 2005). Since the overwhelming inflammatory and immune response during the early stage of sepsis involves a vast array of mediators, controlling this complex inflammatory cascade is critical for sepsis management (Glauser et al., 1994). Thus, natural products that have various components that act on different cascades are likely to be more beneficial for sepsis treatment than drugs targeting a
single mediator. Aloe vera has been used therapeutically for many centuries and is of particular interest due to its lengthy historic reputation as a curative agent and its widespread use in complementary therapies (Reynolds and Dweck, 1999). Polysaccharides isolated from the gel of Aloe vera, acemannan, have also been shown to completely cure or significantly reduce tumor burden (Peng et al., 1991), to increase lymphocyte responses to alloantigens in vitro (Wombles and Helderman, 1992), to increase the NO production by macrophages (Karaca et al., 1995), and to up-regulate the phagocytic and bacterial activities of macrophages (Stuart et al., 1997). Here, we demonstrated the protective effect of Aloe vera on animal model of sepsis, i.e. lethality induced by CLP.

The CLP model of peritonitis and sepsis has been a mainstay of basic sepsis research. Several experimental studies showed that CLP causes inoculation of colonic content into the peritoneal cavity and results in episodic bacteremia and systemic changes such as hyperpyrexia, leukocytosis and tachycardia. These also reflect the progress of clinical human sepsis that occurs as a consequence of invasion of the body by gram-negative or gram-positive bacteria, fungi, and, probably, viruses and parasites. In our study, Aloe vera effectively reduced CFUs in the peritoneal cavity in CLP-induced septics animals, and this result enabled us to suggest that Aloe vera could be used as an antiSeptic agent.

In addition to its bacterial effect, Aloe vera reduced lethality in septic animals. Since we have used the moderate CLP model, 10-day survival studies allowed us to clearly observe the pharmacotherapeutic effect of Aloe vera. In CLP groups, the survival rate decreased continuously for 5 days, and on the sixth day, the rate plateaued. From the first day after CLP operation, Aloe vera effectively reduced CFUs in the peritoneal cavity in CLP-induced septics animals, and this result enabled us to suggest that Aloe vera could be used as an antiSeptic agent.

As a consequence of an overactive response to an infection, systemic inflammatory response syndrome (SIRS), a general term for sepsis, can compromise the function of distinct organ systems, leading to MODS (Matsuda and Hattori, 2006). When SIRS results in MODS and organ failure, the mortality becomes high and can be more than 50% (Brun-Buisson, 2000). Organ failure often begins with respiratory failure, followed by intestinal, hepatic, renal, hemolactoytic, and cardiac failure; the exact order may vary because of preexisting disease or the precipitating insult (Deitch, 1992). Mortality is strongly correlated with the number of organ systems failing, as well as age and duration of organ failure (Ahmed et al., 1995). A definite explanation for the pathophysiology of MODS has yet to be elucidated. In the present study, histological analyses were performed to examine the effect of Aloe vera on MODS. In heart, kidney, liver, and lung samples, CLP induced marked histopathological changes such as congestion, inflammatory cell infiltration, necrosis, and degeneration compared to sham operation. These changes were ameliorated by Aloe vera treatment. These morphological improvements with Aloe vera treatment were also confirmed by assessing biochemical parameters. We examined the degree of heart, renal, and liver dysfunction by monitoring serum levels of LDH, BUN, creatinine, and ALT for 48 h after CLP. The levels of LDH, BUN, and creatinine peaked 12 h after CLP operation, while the peak level of ALT appeared at 24 h after CLP. This time-dependent CLP-induced enzyme profile may indicate differences in the vulnerabilities of various organs affected by sepsis. These increases were significantly attenuated by Aloe vera treatment, which provided significant protection from acute organ dysfunction.

Previous studies have demonstrated that TNF-α, IL-1β, IL-6, and IL-8 are the most strongly associated cytokines with sepsis syndrome (Blackwell and Christman, 1996; Takala et al., 2002). TNF-α and IL-1β are the most powerful pathological cytokines, causing cell degeneration, apoptosis and necrosis and leading to multiple organ dysfunction (Tracey et al., 1986). And besides, as proximal cytokines, TNF-α and IL-1β also stimulate the production of later or distal cytokines, such as IL-6 and IL-8. Recently, Chen et al. (2001) suggested that TNF-α and/or IL-1β are essential initiators of septic response in the liver, and IL-6, a second-phase cytokine, is more likely to mediate dysfunction and death in late sepsis. Therefore, interfering with the cytokine overproduction during early sepsis may improve sepsis outcome. In our study, TNF-α and IL-1β levels dramatically increased in serum by 3 h after CLP and decreased by 48 h after CLP, showing their short and sharp secretion pattern. These results were also supported by data from several other groups including Tracey et al. (1987). Treatment with Aloe vera significantly inhibited the elevation of both TNF-α and IL-1β levels. Similar to TNF-α and IL-1β, IL-6 contributes to the pathogenesis and progression of sepsis. However, Remick et al. (2005) also demonstrated that complete lack of IL-6 has limited impact on the overall mortality in a standardized animal model of sepsis. Our results show that the serum IL-6 level was significantly attenuated by Aloe vera treatment in septic animals.

In conclusion, Aloe vera has potent therapeutic effects in polymicrobial sepsis by decreasing pro-inflammatory cytokines associated various cytokine cascades in sepsis pathogenesis and ultimately leading to the alleviation of MODS. Thus, we propose that Aloe vera is a potential therapeutic medication for the care of clinical septic patients.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References


