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(RESEARCH ARTICLE)

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Barrier-protective properties of pterostilbene against LPS-induced sepsis

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Abstract

Background: Sepsis is a major clinical problem and the leading cause of death in patients in intensive care units worldwide.

Objective: The present study aimed to explore whether pterostilbene (PTS) could protect mice against experimental endotoxemia.

Methodology: Forty-eight mice were randomly divided into 4 groups and challenged with LPS. Mouse mortality was observed twice daily for 7 days and survival rates were reported. Mice were randomly treated with pterostilbene or vehicle intraperitoneally (i.p.). One hour later, the animals were exposed to LPS (20 mg/kg). Cytokine responses were then assessed in serum isolated from blood collected after LPS administration to the mice.

Results: The results showed that pterostilbene significantly reduced mouse body weight loss and attenuated inflammatory responses by inhibiting TNF-α, IL-6 and IL-1β production in mice exposed to LPS.

Conclusion: This study highlights the role of pterostilbene in the pathogenesis of LPS-induced sepsis and its potential in the treatment of patients with sepsis.

Keywords: Sepsis; Inflammatory Responses; Pterostilbene; Mice

1. Introduction

Sepsis is a major clinical problem and the leading cause of morbidity and mortality in modern intensive care units. Several studies have provided epidemiological data on sepsis in patients in the developed world [1-6]. The septic shock is characterized by an excessive inflammatory response associated with high mortality [7-9].

Macrophages are one of the key types of cells involved in the pathogenesis of sepsis and exert effects by secreting inflammatory cytokines [10]. Lipopolysaccharide is the main constituent of the outer membrane of Gram-negative bacteria and is a key pathogen-associated molecular pattern in septic shock. It promotes septic shock via the activation of macrophage cell surface receptor Toll-like receptor 4 (TLR4), which leads to the secretion of proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) [11-14]. A better understanding of sepsis pathology and the development of new therapies are essential to meaningfully improve the status of sepsis care.

Natural products from traditional Chinese medicines constitute a significant portion of the pharmaceutical market today owing to their numerous biological activities. Polyphenols are bioactive compounds that extensively existed in plant

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foods and have many health-promoting effects by the different mechanisms, such as antioxidation, anti-inflammation, immunomodulation, and modulating gut microbiota [15-17].

Pterostilbene (PTS, **figure 1**) is a representative of stilbene compounds that can be found naturally in blueberries and red sandalwood [18]. It has been reported as an effective antioxidant and anti-inflammatory agent. The effects of pterostilbene have been known to ameliorate the immediate inflammatory responses in TNFα (tumor necrosis factor alpha)-induced pancreatitis through down-regulation of Stat3 and the secretion of lipase and inflammatory cytokines IL-1β (interleukin-1 beta) and IL-6 (interleukin-6) [19-20]. In another cell model, pterostilbene down-regulates inflammatory iNOS and COX-2 gene expression in macrophages by inhibiting NFκB activation through interfering with the activation of PI3K/Akt/IKK (IκB kinase) and MAPK [21]. Furthermore, many studies have shown that pterostilbene can suppress the expression of several genes and their inflammatory products in LPS -stimulated RAW 264.7 macrophages and peritoneal macrophages from mice. Authors suggest that garcinol and pterostilbene may provide novel and useful applications to reduce the chronic inflammatory properties of adipocytes. In the TNF-α-induced 3T3-L1 adipocyte model, pterostilbene with garcinol suppressed phosphorylation of p-IκBα and p-p65. In a coculture model of 3T3-L1 adipocytes and RAW 264.7 macrophages, it suppressed IL-6 and TNF-α secretion and proinflammatory mRNA expression and also reduced the migration of macrophages toward adipocytes [22]. It has been known that any means to regulate an excessive systemic inflammatory response would be a promising and beneficial strategy leading to a mitigation of endotoxic sepsis. Although research has shown the multispectrum pharmacological benefits of pterostilbene for the treatment of various chronic diseases, its potential effect on septic shock remains unclear. Therefore in this study, we first studied the role of pterostilbene in septic shock and then explored its capacity to provide a survival advantage in the presence of LPS-induced endotoxemia.

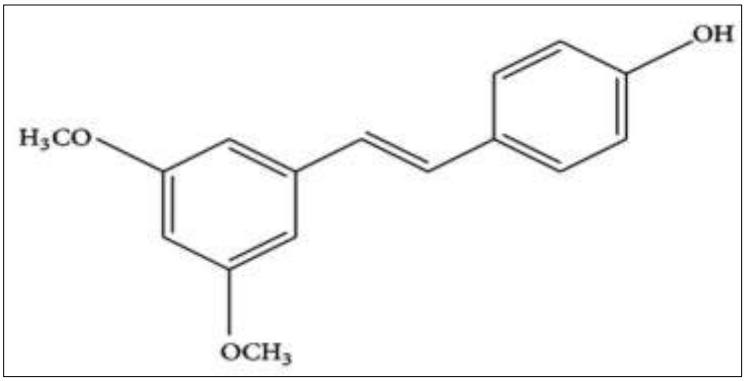


Figure 1 The chemical structure of Pterostilbene

2. Materials and methods

2.1. Chemical and reagents

Pterostilbene (purity ≥97%) was purchased from Sigma-Aldrich (China). Dimethyl sulfoxide (DMSO) and lipopolysaccharide (LPS, Escherichia coli 055:B5) were purchased from Sigma Chemical Co. (San Diego, CA, USA). (TNF)-α, IL-1β, and IL-6 ELISA kits were purchased from Biolegend (San Diego, CA).

2.2. Animals

Eight-week-old male BALB/c mice, weighing approximately 18-20 g, were maintained in an animal facility under pathogen-free conditions. Mice were fed a standard ration and water ad libitum, and housed in micro-isolator cages under standard conditions (temperature: 24 ± 1°C, relative humidity: 40%-80%). The mice were able to adapt to their environment for 2 to 3 days prior to experimentation.

2.3. Induction of shock and measurement of cytokines concentrations

Forty-eight mice were divided into four groups (n=12/group) and given LPS (5-40 mg/kg) via a single intraperitoneal (IP) injection to select an appropriate concentration of LPS to induce shock. Mouse mortality was observed bi-daily for 7 days and survival rates were monitored.

Mice were randomly divided into five groups: control (vehicle), LPS (20 mg/kg) only and Pterostilbene (10, 20 or 40 mg/kg) + LPS. Mice were treated with Pterostilbene at different doses (10, 20 or 40 mg/kg) or vehicle (5% of DMSO in saline), by the intraperitoneal (i.p.) route. Dose selection of Pterostilbene was made based of the previous studies [23-25]. One hour later, the animals were exposed to LPS (20 mg/kg).

Blood was drawn from the tail vein of each mouse at 0, 1, 2, 4 and 8 h after LPS administration, and serum was isolated; serum was stored at -70°C. TNF-α, IL-6 and IL-1 concentrations in serum samples were measured by enzyme-linked immunosorbent assay (ELISA), using the DuoSet kit from R&D Systems (Minneapolis, MN).

2.4. Statistical analysis

Results are presented as means ± SEM. Differences between mean values of normally distributed data were assessed with one-way ANOVA (Dunnett's t-test) and two-tailed Student's t-test. Statistical significance was accepted at $P<0.05$ or $P<0.01$.

3. Results

3.1. Effect of RT on LPS-induced mortality and cytokine responses

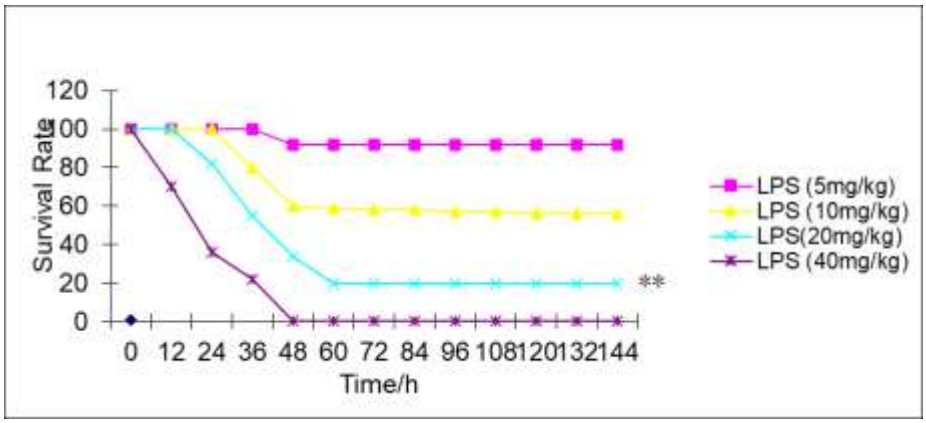


Figure 2 Effects of different doses of LPS in a mouse model of sepsis. The mice were divided into four groups (n=12/group) and challenged with LPS (5–40 mg/kg) via a single intraperitoneal (IP) injection. The mice were observed for mortality twice a day for 7 days, and survival rates were recorded.

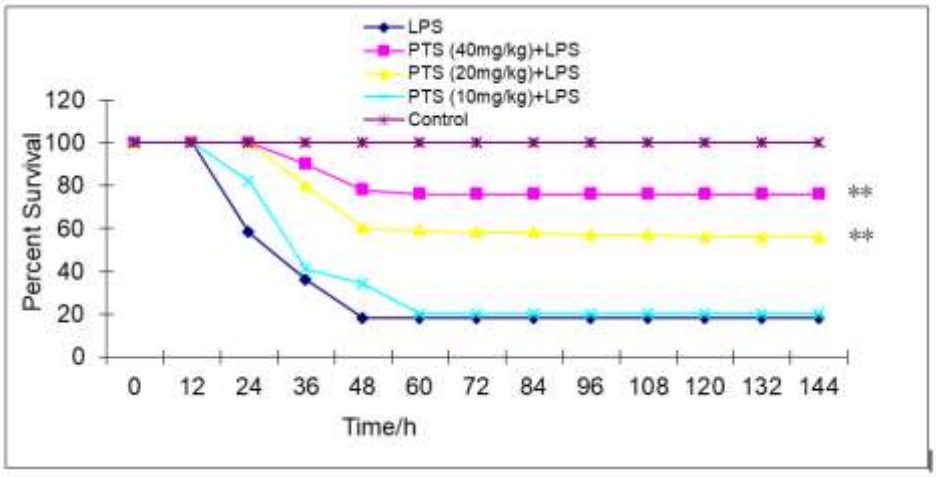
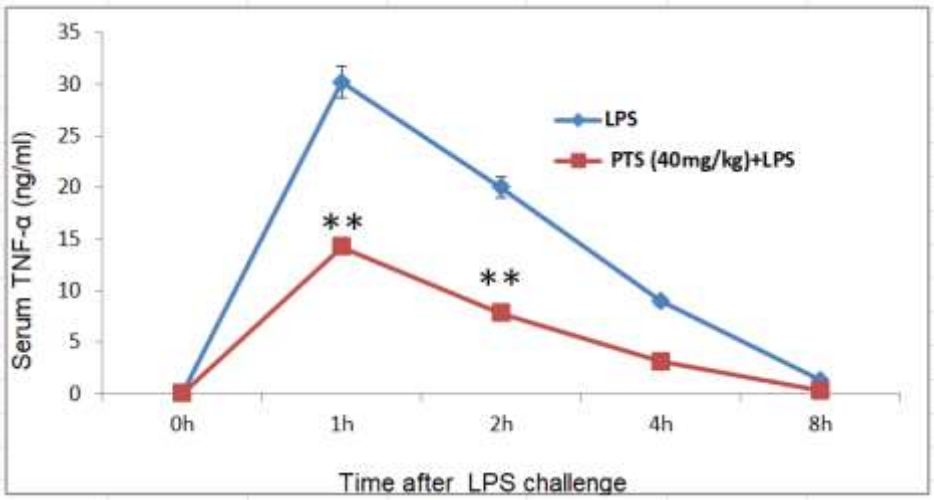
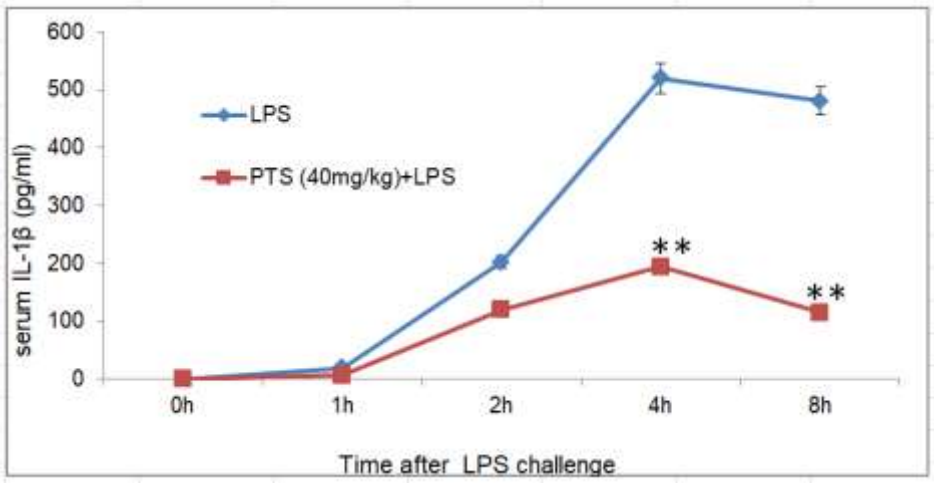


Figure 3 Effect of administration of pterostilbene on LPS-induced sepsis in mice. Mice were divided into five groups (control, LPS (20 mg/kg) only, and LPS (20 mg/kg) + 3 treatment groups of pterostilbene) (n= 12/group). **p<0.01 versus LPS-only group





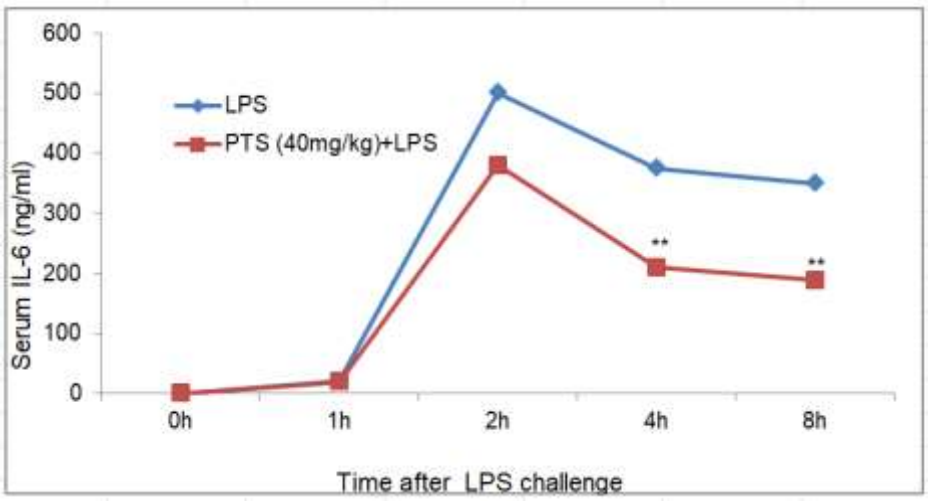


Figure 4 Effects of pterostilbene on the levels of pro-inflammatory cytokines. Mice were injected IP with pterostilbene 1 h before challenge with the LPS (20 mg/kg) and the blood samples were collected from mice 0, 1, 2, 4, and 8 h after LPS challenge. The serum levels of inflammatory cytokines were measured using ELISA. **p<0.01 versus LPS-only group

After LPS injection, the mice exhibited signs of acute septicemia (reduced activity, conjunctivitis, diarrhea, lethargy, piloerection, huddling etc.). In the groups of mice receiving 5, 10 or 20 mg LPS/kg, mortality rates were 5, 18 and 80%

(**Figure 2**), respectively. Therefore, the LPS 20 mg/kg concentration was selected as the lethal dose inducing septic shock. In the following experiments, mice were inoculated with 20 mg LPS/kg.

To evaluate the benefit of pterostilbene against lethal endotoxemia, its role in the mortality of mice affected by this disease was monitored. Consequently, pterostilbene was able to significantly reduce LPS-induced mortality in mice. The minimum dose of pterostilbene showed 34% protective effect. Nevertheless, treatment with 20 or 40 mg/kg pterostilbene prevented the mice from developing lethal experimental sepsis (64% and 89% protective effect, respectively, **Figure 3**).

To assess the effects of pterostilbene, levels of endotoxemia-related pro-inflammatory cytokines were measured in mouse blood samples. The present study revealed that the expression levels of TNF-α, IL-1b and IL-6 in mice pretreated with pterostilbene (40mg/Kg) and challenged with LPS (20mg/Kg) were systematically reduced compared with mice given LPS alone (**figure 4**).

4. Discussion

In the current study, we provide evidence indicating that Pterostilbene is able to inhibit inflammatory cytokines in LPS-challenged mice and improve survival rates of mice in LPS-induced septic shock.

A number of murine models of sepsis, notably the endotoxemia model, the bacterial injection model, and cecal ligation and puncture, have been developed for pathological studies and drug development. The murine LPS injection endotoxemia model is widely employed for experimental investigations of sepsis [26-27]. The research protocol investigating the effect of Pterostilbene has been used to assess the main preventive and curative attributes of the natural product on endotoxemia.

Septicemia is a highly life-threatening organ dysfunction associated with a dysregulated host response to infection. Recognized as a major cause of morbidity and mortality, sepsis and septic shock are major health problems, affecting millions of people worldwide every year and killing between one in three and one in six [28-31]. Our experimental results indicated that treatment with pterostilbene results in the reduction of LPS-induced septic mortality; therefore, our results suggested that pterostilbene can produce ameliorative effects in endotoxemia.

LPS activates Toll-like receptor 4 and eventually nuclear factor-kappa B mechanism followed by the release of inflammatory cytokines, such as TNF-α, IL-1α/β, IL-6, IL-12, IL-18, and GM-CSF. A large proportion of proinflammatory mediators comprising cytokines are responsible for metabolic changes associated with cellular injury. Cytokines are chemical mediators of the immune and acute phase responses. TNF-α, IL-1β, and IL-6 are the major mediators of acute phase response in humans. Additionally, IL-6 functions as an endogenous pyrogen that stimulates the immune system and, in conjunction with TNF-α, can stimulate the synthesis of acute phase proteins [32-34]. Septic syndrome is an acute systemic illness associated with shock, coagulopathy and multi-organ dysfunction. Mortality occurs in between 25% and 35% of patients with this syndrome. Several studies have shown that the pathogenesis of fatal sepsis remains obscure, but is associated with dysregulated induction of inflammatory mediators. The initial manifestations of severe sepsis trigger a cascade of events that ultimately contribute to the morbidity and mortality of patients. More specifically, compared with the control group, we observed a significant upregulation of endotoxin-induced mortality in LPS-treated subjects. Moreover, pre-administration of Pterostilbene (20 mg/kg or 40 mg/kg) was found to provide considerable protection [35-40]. In the event of sepsis, the macrophages induce pro-inflammatory cytokines such as interleukin (IL)-1β, which trigger an innate immune response to pathogens. Initiated by pathogens and cytokines, macrophages differentiate into various functional phenotypes and perform different functions, including pathogen elimination, cytokine and chemokine production [41-42]. However, Pterostilbene was observed to be effective in reducing inflammatory mediators. As our results show, Pterostilbene exhibits a protective role against LPS-induced septic shock via inhibition of these cytokines.

5. Conclusion

In this study, our experimental results demonstrated that pterostilbene reduced the LPS-induced mortality of septic mice, inhibited the production of proinflammatory cytokines, and ameliorated the symptoms and pathology associated with sepsis, suggesting that pterostilbene might have benefits for treating the life-threatening organ dysfunction.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Statement of ethical approval

Experiments involving animals were conducted in accordance with experimental practices and standards approved by the university's Research Ethics and Animal Welfare Committee.

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