Original Research Article

Procyanidin A1 improves sepsis-induced liver injury by inhibiting inflammation

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Abstract

Purpose: To determine the effect of procyanidin A1 (PCA1) on sepsis.

Methods: Dulbecco’s Modified Eagle Medium (DMEM) was employed to incubate mouse hepatic cell line AML12. The AML12 cells treated with lipopolysaccharide (LPS, 50 μg/mL) was used to establish a sepsis cell model. Cell viability was evaluated using CCK-8 assay, while cell apoptosis was assessed by flow cytometry. Aspartate transaminase (AST), alanine aminotransferase (ALT), IL-6 and TNF-α levels were evaluated by enzyme linked immunosorbent assay (ELISA). Protein expressions were assessed using western blot assay.

Results: The viability of AML12 cells decreased following treatment with IL-1β, but this change was offset by PCA1 treatment (40 or 80 μM). Similarly, cell apoptosis was enhanced after LPS treatment, but this change was attenuated by PCA1 treatment. The AST, ALT, IL-6 and TNF-α levels were all elevated after LPS treatment, but these changes were also reversed by PCA1 treatment, indicating that PCA1 suppressed LPS-induced liver injury and inflammation. Furthermore, the protein levels of p-p65/p65 and p-IκBα increased, and IκBα lowered following LPS treatment, but these effects were reversed by PCA1 treatment, indicating that PCA1 retarded NF-κB pathway.

Conclusion: PCA1 alleviates sepsis-induced liver injury by inhibiting inflammation through NF-κB pathway. This suggests that PCA1 may be a therapeutic agent for the treatment of sepsis.

Keywords: Procyanidin A1, Sepsis, Liver injury, Inflammation, NF-κB pathway

INTRODUCTION

Sepsis is featured by organ dysfunction mediated by a host's dysfunctional response to infection, threatening human life and often resulting in disability and death [1]. Strikingly, the incidence of sepsis in older people is relatively high. Furthermore, sepsis has a worse prognosis in older patients, with higher rates of grievous organ dysfunction, disability, and mortality [2]. The pathogenesis of sepsis leading to grievous organ dysfunction (especially liver injury) and death is incompletely understood. Therefore, it is needful to look for novel and effective drugs to mitigate inflammation and liver damage triggered by sepsis.
Procyanidins are a kind of polyphenolic compounds existing in most plants, which have some pharmacological effects including anti-inflammation, anti-apoptosis and anti-oxidation [3]. As one of the isolated components from procyanidins, procyanidin A1 (PCA1) has attracted the attention of many researchers. Previous studies showed that PCA1 has antioxidant, immunomodulatory and cholesterol regulation effects. For example, PCA1 affects NF-κB, MAPK, and Nrf2/HO-1 pathways in order to reduce the LPS-stimulated inflammation in RAW264.7 cells [4]. Moreover, PCA1 modulates autophagy to relieve DSS-triggered ulcerative colitis [5]. It also regulates MAPK/MLCK signaling pathway in order to ameliorate acrylamide-mediated intestinal barrier dysfunction [6]. Besides, in mice-immunized with ovalbumin, PCA1 cuts down IgE and IgG1 levels [7]. Nevertheless, its effect on the progression of sepsis has not been investigated, and its regulatory impact remains vague.

The NF-κB pathway has been shown to participate in the progression of sepsis [8-10]. However, whether PCA1 modulates the NF-κB pathway to attenuate sepsis progression remains unclear. This study’s goal was to examine the regulatory functions of PCA1 on the progression of sepsis.

**EXPERIMENTAL**

**Cell line and treatment**

Mouse hepatic cell line AML12 was acquired from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and cultivated in Dulbecco’s Modified Eagle Medium (DMEM, Thermo Fisher, Shanghai, China) including 10% fetal bovine serum (FBS, Gibico, USA) and 1% penicillin/streptomycin in a wet incubator (5% CO₂, 37 °C).

The AML12 cells treated with LPS (50 μg/mL) was used to construct a sepsis cell model. PCA1 (0, 20, 40, 80, 100 μM; Chengdu Push Biotechnology) was used to treat the AML12 cells.

**CCK-8 assay**

Cell viability was tested using Cell counting kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). The AML12 cells (1 × 10⁴ cells/well) were placed in a 96-well plate for 48 h. Then, each well was incubated with CCK-8 solution (10 μL) for another 4 h. Next, a spectrophotometer (Thermo Fisher Scientific, MA, USA) was used to assess the absorbance at 450 nm wavelength.

**Flow cytometry**

Cell apoptosis was evaluated using Annexin-V-PI Apoptosis Detection kit (BD Biosciences, Franklin Lakes, NJ, USA). Staining using 5 μL Annexin V-fluorescein isothiocyanate (FITC, 50 μg/mL) and counterstaining using 5 μL propidium iodide (PI, 50 μg/mL), were done on the re-suspended AML12 cells in a dark room. Lastly, cell apoptosis was measured in a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

**Enzyme linked immunosorbert assay (ELISA)**

Aspartate transaminase levels (AST, ab263882, Abcam, Shanghai, China), alanine aminotransferase (ALT, ab282882), interleukin-6 (IL-6, ab222503), and tumor necrosis factor-α (TNF-α, ab208348) were evaluated using commercial ELISA kits based on the kit manufacturer’s instructions.

**Western blot**

The proteins isolated from AML12 cells were analyzed using RIPA lysis buffer. Next, the separation of proteins was done using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and were migrated onto the polyvinylidene difluoride membrane (PVDF, Sigma, St Louis, USA). Addition of primary antibodies onto the membranes were carried out for one night, next for secondary antibody (1:2000; ab6721) for 2 h. Finally, an enhanced chemiluminescence system (Thermo Fisher Scientific, USA) was utilized for evaluating the protein bands. The primary antibodies used include p-p65 (1:1000; ab76302), p65 (0.5 μg/mL; ab16502), p-IkBα (1:10000; ab133462), IkBα (1:1000; ab32518) and β-actin (1 μg/mL; ab8226).

**Statistical analysis**

The data were presented as mean ± standard deviation (SD), while statistical analysis was carried out using SPSS 20.0 (IBM Corp, Armonk, NY, USA). Each experiment for at least 3 times was repeated. The analysis of differences were done by Student’s t-test (two groups) or one-way ANOVA (multiple groups). P < 0.05 was regarded as statistically significant.

**RESULTS**

**PCA1 enhanced LPS-stimulated cell viability**

The molecular structure of PCA1 is presented in Figure 1 A. Cell viability was not changed after PCA1 treatment at concentrations < 80 μM, but
decreased after PCA1 treatment at concentration of 100 μM (Figure 1 B). Then, the 20, 40, 80 μM of PCA1 were selected for further experiments. When AML12 cells stimulated by LPS (50 μg/mL) treatment were used to establish the cell model, cell viability decreased after LPS treatment, but this effect was reversed by PCA1 treatment (40 or 80 Mm; Figure 1 C). Thus, PCA1 enhanced LPS-triggered cell viability.

**PCA1 attenuated LPS-stimulated cell apoptosis**

Cell apoptosis was heightened after LPS treatment, but this effect was attenuated by PCA1 treatment (40 or 80 Mm; Figure 2). Thus, PCA1 attenuated LPS-stimulated cell apoptosis.

**PCA1 suppressed LPS-triggered liver injury and inflammation**

The hepatocyte injury markers, AST and ALT, were both elevated after LPS treatment, but these effects were offset by PCA1 treatment (40 or 80 Mm; Figure 3 A). Moreover, the levels of the inflammation markers, IL-6 and TNF-α, were both enhanced after LPS treatment, but these changes were reversed by PCA1 treatment (40 or 80 Mm; Figure 3 B). Thus, PCA1 suppressed LPS-induced liver injury and inflammation.

**PCA1 inhibited NF-κB pathway**

The p-p65/p65 and p-IκBα levels increased, and IκBα levels decreased after LPS treatment, but these changes were reversed by PCA1 treatment (40 or 80 Mm; Figure 4). Thus, PCA1 inhibited NF-κB pathway.
DISCUSSION

Lately, traditional Chinese medicines have been found to play an increasingly pivotal role in the treatment of sepsis. For instance, 6-gingerol regulates the Nrf2 pathway in order to improve sepsis-mediated liver injury [11]. In addition, paecanol modulates mitochondrial function and NF-κB translocation so as to relieve LPS-induced hepatocyte injury [12]. Paclitaxel targets miR-27a/TAB3/NF-κB signaling pathway in septic mice in order to improve liver injury via the alleviation of inflammatory responses [13]. Furthermore, baicalin represses inflammation and apoptosis thereby attenuating sepsis-triggered liver injury [14]. PCA1 has been shown to have regulatory influences on some diseases [4-7], but its role in sepsis remains unclear. In the present study, it was observed that the viability of AML12 cells was reduced after LPS treatment, but this change was offset by PCA1 treatment (40 or 80 μM). Similarly, cell apoptosis was enhanced after LPS treatment, but this effect was attenuated by PCA1 treatment.

Many cytokines are secreted during sepsis, and this is associated with the severity of this disease [15]. TNF-α and IL-6 are the principal inflammatory mediators, and their levels are significantly elevated in sepsis. The liver is a vital organ in the development of sepsis, and is both the source of these cytokines and a target organ for these mediators that cause liver injury [16]. Inflammation is a crucial process in sepsis [17]. In this study, it was discovered that the AST, ALT, IL-6 and TNF-α levels were all elevated after LPS treatment, but these effects were countered by PCA1 treatment, indicating that PCA1 suppressed LPS-induced liver injury and inflammation.

Excessive production of cytokines is modulated by a variety of signaling pathways, including nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) [18]. During sepsis, the binding activity of NF-κB, the degradation of the inhibitor of NF-κB (IκB), and the nuclear accumulation of NF-κB are all strengthened. Therefore, retardation of the activation of NF-κB suppresses the generation of inflammatory cytokines and liver injury [19]. The NF-κB pathway has been revealed as playing a role in the progression of sepsis [8-10]; however, whether PCA1 modulates NF-κB pathway thereby ameliorating sepsis remains unknown. In this study, the protein levels of p-p65/p65 and p-IκBa increased, and that of IκBa decreased after LPS treatment, but these changes were reversed by PCA1 treatment, indicating that PCA1 inhibited NF-κB pathway.

CONCLUSION

To the best of our knowledge, this is the first study to demonstrate that PCA1 alleviates sepsis-induced liver injury by enhancing cell proliferation, as well as by inhibiting cell apoptosis and inflammation via NF-κB pathway. Thus, PCA1 is a potential drug for the treatment of inflammation and liver damage triggered by sepsis, but this needs to be investigated in in vivo studies.

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Ethical approval
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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest
No conflict of interest associated with this work.

Contribution of Authors
The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Zhan Yao, Shuna Liu designed the study and carried it out; Zhan Yao, Shuna Liu, Chunmei Zheng, Qiangwu Li, and Liya Wang supervised the data collection, analyzed and interpreted the data; and Zhan Yao and Shuna Liu prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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