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Therapeutic effects of α-iso-cubebenol, a natural compound isolated from the *Schisandra chinensis* fruit, against sepsis

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ABSTRACT

α-Iso-cubebenol, a natural compound isolated from the *Schisandra chinensis* fruit, strongly enhances survival rate in cecal ligation and puncture (CLP) challenge-induced sepsis. Mechanistically, α-iso-cubebenol markedly reduces viable bacteria in the peritoneal fluid and peripheral blood, by increasing production of superoxide anion. α-Iso-cubebenol also significantly attenuates widespread immune cell apoptosis in a mouse CLP sepsis model, and inhibits the production of proinflammatory cytokines including interleukin-1β (IL-1β) and IL-6 in CLP mice and lipopolysaccharide-stimulated splenocytes. Taken together, the results indicate that α-iso-cubebenol can reverse the progression of septic shock by triggering multiple protective downstream signaling pathways to enhance microbial killing and maintain organ function and leukocyte survival.

1. Introduction

Sepsis, a systemic inflammatory response, is caused by viable bacteria or bacterial products such as lipopolysaccharide (LPS) [1]. More than 750,000 patients develop sepsis annually in the United States, and the incidence rate is gradually increasing [2]. The number of hospitalizations with sepsis in the US increased from 300,000 to around 800,000 from 2000 to 2007 [3]. Although the mortality rate of sepsis decreased somewhat from 2000 to 2007, sepsis remains the major cause of death in intensive care units, and the overall mortality associated with sepsis ranges from 30% to 70% [2]. Only 2% of hospitalizations are for sepsis, yet it makes up 17% of in-hospital deaths in the US [4]. Sepsis-induced lethality is accompanied by the failure of an appropriate immune response against invading pathogens [5,6]. The inability of the innate immune system to respond during early sepsis (i.e., the first 6 h) results in increased mortality. Excessive lymphocyte apoptosis can occur during sepsis, which results in the clinical signs of multi-organ failure [7,8]. Since sepsis is caused by viable bacteria or bacterial products, the levels of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-1β are substantially increased during sepsis [9–11]. Thus, an effective treatment for sepsis should control bacteria, prevent production of inflammatory cytokines, and block widespread leukocyte apoptosis.

We previously isolated a novel natural compound, α-iso-cubebenol, from the *Schisandra chinensis* fruit and demonstrated that the compound inhibits inducible nitric oxide synthase and cyclooxygenase-2 expression in lipopolysaccharide (LPS)-stimulated macrophages [12]. Subsequently, α-iso-cubebenol has been reported to induce heme oxygenase-1 expression and have anti-inflammatory activity in *Porphyromonas gingivalis* LPS-stimulated macrophages [13]. In this study we investigated the *in vivo* efficacy of α-iso-cubebenol in a preclinical mouse model of sepsis. We also examined the mechanisms of septic protection by this novel natural compound.

2. Materials and methods

2.1. Purification of α-iso-cubebenol

α-Iso-cubebenol (CAS registry number: 1219105-52-8) was purified from the dried fruit of *S. chinensis* as described previously [12]. The fruit of *S. chinensis* (Turcz.) Baill was collected in September 2005 from Moonkyong, Korea. A voucher specimen (Accession

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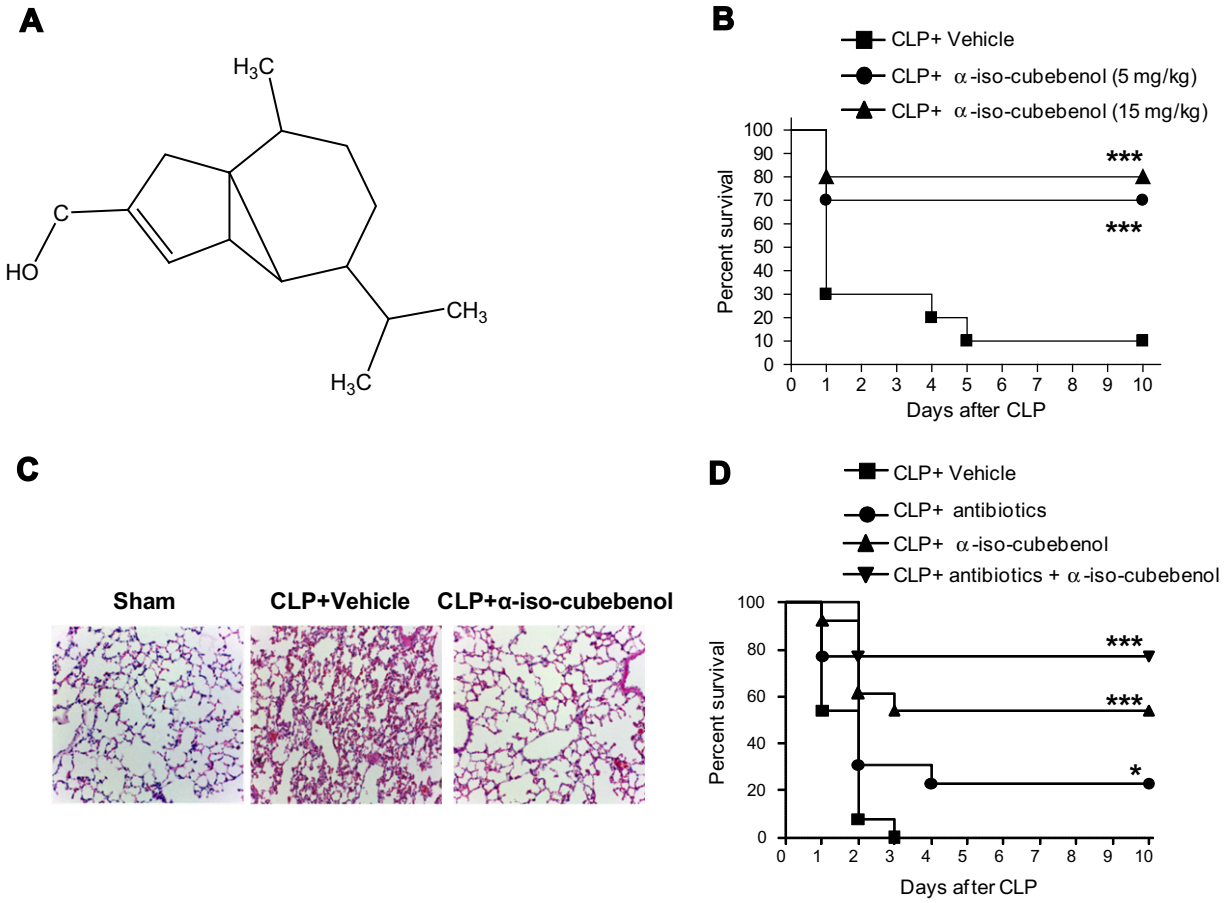

Fig. 1. α-Iso-cubebenol protects against CLP-induced mortality. (A) The chemical structure of α-iso-cubebenol. (B) Several doses (0, 5, 15 mg/kg) of α-iso-cubebenol were injected subcutaneously four times into CLP mice at 2, 14, 26, and 38 h post-CLP. (C) Vehicle (0.8% DMSO in PBS) or α-iso-cubebenol (15 mg/kg) was administered 2 and 14 h after CLP. The mice were sacrificed 24 h after surgery and the lungs stained with hematoxylin and eosin (magnification, 100×). (D) Vehicle (0.8% DMSO in PBS), α-iso-cubebenol (15 mg/kg) or antibiotics (10 mg/kg gentamycin plus 10 mg/kg cephalosporin), or α-iso-cubebenol plus antibiotics were injected subcutaneously four times into CLP mice at 2, 14, 26, and 38 h post-CLP. $^{*}P < 0.05$, $^{***}P < 0.001$ compared to vehicle control by ANOVA (B and D). Sample size: $n = 10$ mice/group (B) or 13 mice/group (D). The data are representative of 8 mice/group (C).

No. SC-PDRL-1) has been deposited in the Herbarium of Pusan National University. The plant was identified by one of the authors (Y. Choi). The dried fruit of *S. chinensis* (2.5 kg) ground to a fine powder and then successively extracted at room temperature with *n*-hexane, $CHCl_3$, and MeOH. The hexane extract (308 g) was evaporated *in vacuo* and chromatographed on a silica gel (40 μm; Baker, Phillipsburg, NJ, USA) column (100 × 10 cm) with a step gradient of EtOAc in hexane (0%, 5%, and 20%) and 5% MeOH in $CHCl_3$ to obtain 38 fractions as described [14]. Fraction 9 (KH9, 4866 mg) was separated on a silica gel column (100 × 3.0 cm) with a step gradient (1%, 10%, and 15%) of acetone in $CHCl_3$ to obtain 21 fractions. Of these 21 fractions, fraction 2 (KH9IG, 529.9 mg) was separated on a silica gel column (100 × 3.0 cm) with 5% acetone in $CHCl_3$ to yield α-iso-cubebenol (162.9 mg). Pure α-iso-cubebenol was identified by HPLC on a Phenomenex Luna C18 column (150 × 4.6 mm internal diameter, 5 μm particle size; Phenomenex, Los Angeles, CA, USA) with a methanol–acetonitrile gradient, at a flow rate of 1.0 ml/min. The purity of α-iso-cubebenol was over 96%.

2.2. Animals and sepsis model

Six week old male wild type ICR mice were used as an experimental sepsis model as described [15]. For cecal ligation and puncture (CLP), mice were anesthetized with intraperitoneal injections of Zoletil (50 mg/kg) and Rompun (10 mg/kg), after which a small abdominal midline incision was made to expose the cecum. The cecum was then ligated below the ileocecal valve, punctured twice through both surfaces (or once for measurement of cytokine production) using a 22-gauge needle, and the abdomen was closed. Sham CLP mice were subjected to the same procedure but without ligation and puncture of the cecum. Survival was monitored once daily for 10 days.

2.3. Measurement of bactericidal activity in vivo

Twenty-four hours after CLP, peritoneal lavage fluid and peripheral blood were collected and cultured overnight on blood-agar base plates (Trypticase Soy Agar Deeps; Becton Dickinson, Franklin Lakes, NJ, USA) at 37 °C. Colony forming units (CFUs) were determined as described previously [15].

2.4. Measurement of phagocytic activity

Raw 264.7 cells (2×10^5) were resuspended in 100 μl phosphate-buffered saline (PBS) containing 1% human AB serum and 0.02% sodiumazide, preincubated with or without α-iso-cubebenol for 30 min. The cells were further incubated with FITC-dextran (1 mg/ml) at 37 °C for 30 min. The incubations were stopped by adding 2 ml ice-cold PBS containing 1% human serum. After fixing the cells with 3.7% formaladehyde, phagocytic uptake was analyzed on a flow cytometer (FACS Canto II).

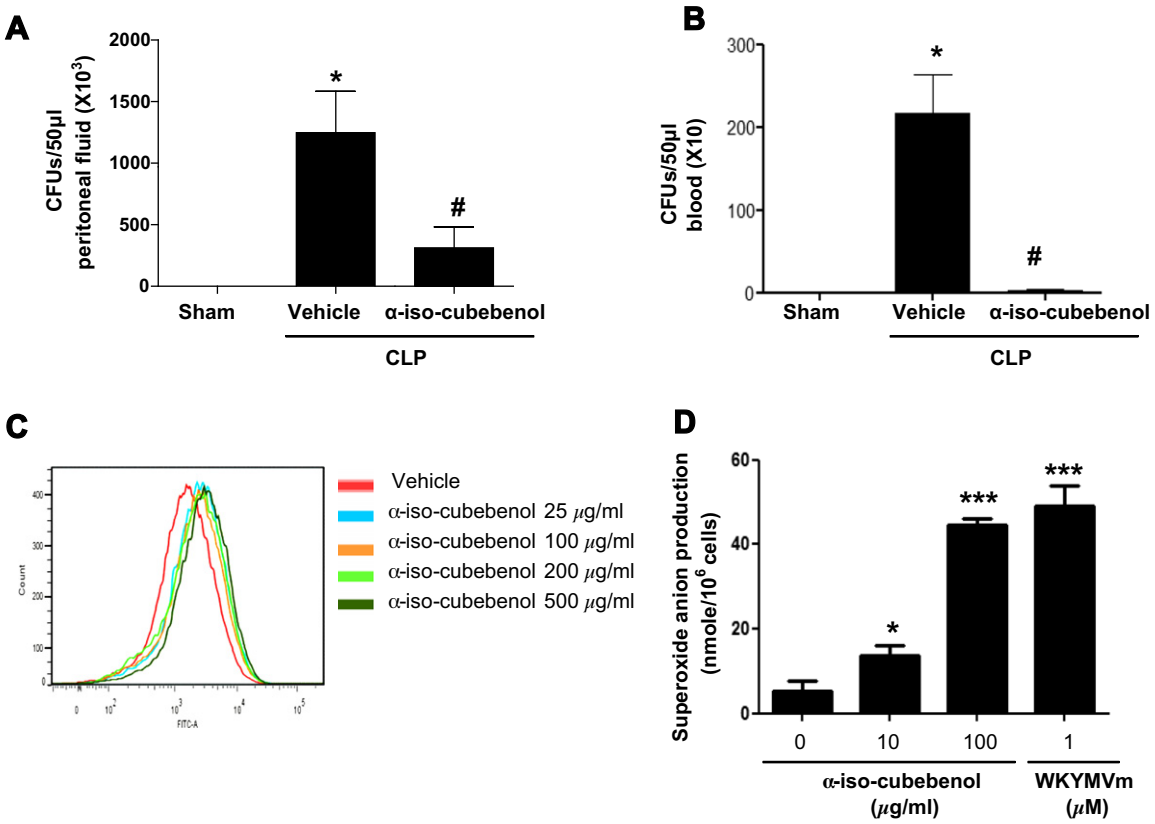

Fig. 2. α-Iso-cubebenol stimulates bactericidal activity. (A and B) Vehicle (0.8% DMSO in PBS) or α-iso-cubebenol (15 mg/kg) was injected two times into CLP mice 2 and 14 h post-CLP. Peritoneal lavage fluid (A), or peripheral blood (B) collected 24 h after sham operation, CLP, or CLP plus α-iso-cubebenol administration was cultured overnight on blood-agar base plates at 37 °C, and CFUs were determined. (C) Raw 264.7 cells (2×10^5) were resuspended in 100 μl PBS and preincubated with or without α-iso-cubebenol for 30 min. Then the cells were further incubated with FITC-dextran (1 mg/ml) at 37 °C for 30 min. After fixing the cells, phagocytic uptake was analyzed on a flow cytometer. The result is representative of three independent experiments. (D) Freshly isolated human neutrophils were stimulated with vehicle (0.1% DMSO in PBS) or α-iso-cubebenol (10 or 100 μg/ml) for 5 min. The amount of superoxide anions produced from neutrophils was measured using a cytochrome c reduction assay. Data are expressed as the mean ± SEM; $n = 8$. *, $P < 0.05$, compared with the value obtained from the sham control (A, B); #, $P < 0.05$, significantly different from the CLP alone control (A, B). *$P < 0.05$; ***$P < 0.001$ compared to the vehicle control (D).

2.5. Measurement of superoxide anion production

Superoxide anion generation was determined by measuring cytochrome c reduction using a microtiter 96-well plate ELISA reader (Bio-Tek instruments, EL312e, Winooski, VT, USA), as previously described [16]. Human neutrophils were isolated according to the standard procedures of dextran sedimentation, hypotonic lysis of erythrocytes, and use of a lymphocyte separation medium gradient as described previously [15]. The isolated human neutrophils were used promptly. Human neutrophils (2×10^6 cells in RPMI 1640 medium) were preincubated with 50 μM of cytochrome c at 37 °C for 5 min and subsequently incubated with each stimulant. Superoxide generation was determined by measuring light absorption changes at 550 nm over 5 min, at 1 min intervals.

2.6. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

Mice were subjected to CLP surgery and given vehicle (0.8% DMSO in PBS) or α-iso-cubebenol at a dose of 15 mg/kg 2 and 14 h post-surgery. The mice were euthanized 24 h after surgery, after which their spleens and thymuses were isolated. The TUNEL assay was performed in frozen tissue sections using a standard histological protocol. The sections were permeabilized with Triton X-100 at 4 °C for 2 min and flooded with terminal deoxynucleotidyl transferase and digoxigenin-dUTP reaction buffer (TUNEL) reagent for 60 min at 37 °C. The percentage of apoptotic (TUNEL-positive) cells was determined by counting 500 splenocytes under a light microscope [17].

2.7. Immunohistochemistry

Immunohistochemistry was conducted using frozen tissue sections using a standard histological protocol. After incubation with primary antibodies against cleaved caspase-3 (Cell Signaling Technology, Danvers, MA, USA), all sections were stained with horseradish peroxidase-conjugated secondary antibody.

2.8. Cytokine measurement

To measure CLP-induced cytokine production in peritoneal lavage fluid, mice were given α-iso-cubebenol at 2 and 14 h after CLP. Peritoneal lavage fluid was collected at 24 h post-CLP, and the cytokines present in the peritoneal fluid were measured by enzyme-linked immunosorbant assay (ELISA; BD Biosciences Pharmingen, San Jose, CA, USA).

2.9. Cytokine release from splenocytes in vitro

Mouse splenocytes (3×10^6 cells/0.3 ml) were placed in RPMI 1640 medium containing 5% fetal bovine serum (FBS) in 24-well plates and kept in a 5% CO_2 incubator at 37 °C. Mouse splenocytes were stimulated with PBS or LPS (100 ng/ml). After 15 min, cells were subsequently stimulated with vehicle (0.1% DMSO in PBS)

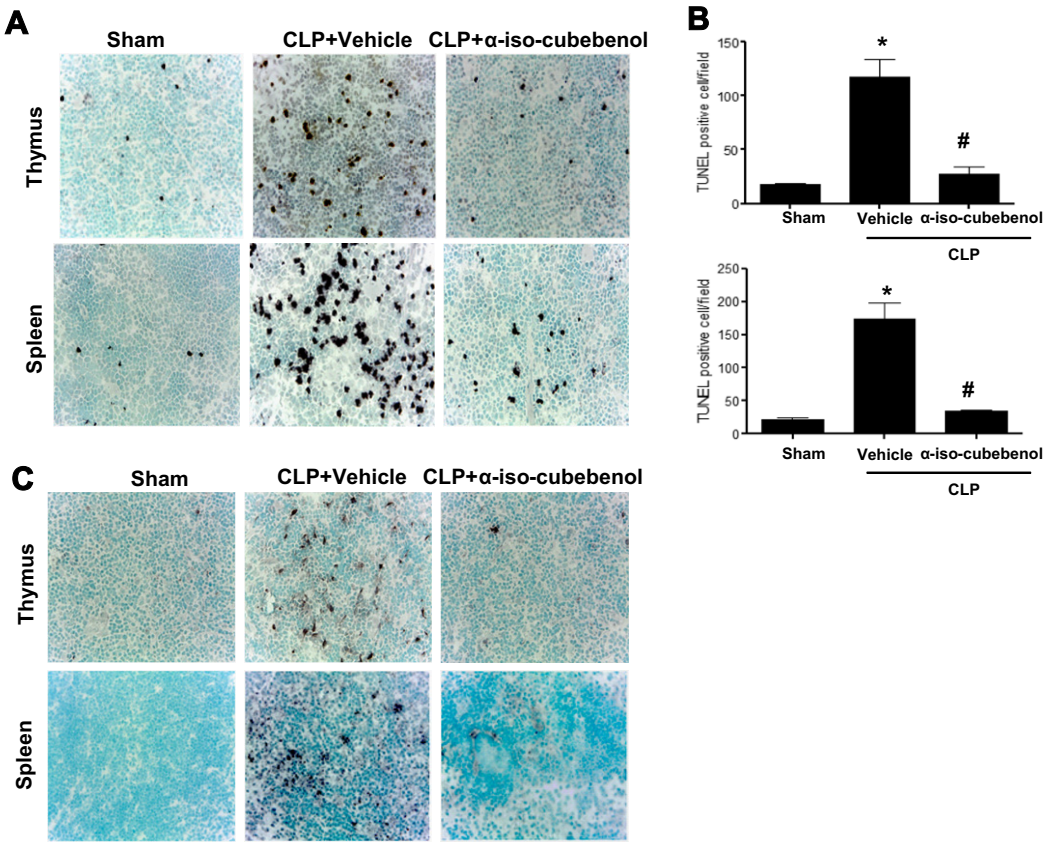

Fig. 3. α-Iso-cubebenol protects against widespread CLP-induced leukocyte apoptosis. (A) Vehicle (0.8% DMSO in PBS) or α-iso-cubebenol (15 mg/kg) was injected two times into CLP mice 2 and 14 h post-CLP. The spleens and the thymuses were collected 24 h after sham, CLP plus vehicle, or CLP plus α-iso-cubebenol administration, and used for a TUNEL assay. (B) TUNEL-positive cells from the spleens and thymuses of mice described in (A) were quantified. Data are expressed as the mean ± SEM ($n = 8$). $^{*}P < 0.05$, compared with the value obtained from the sham control; $^{\#}P < 0.05$, significantly different from the CLP alone control. (C) The spleens and thymuses from the mice described in (A) were subjected to immunohistochemistry with cleaved-caspase-3 antibody (magnification, 100×). Data are representative of eight mice per group (A and C).

or α-iso-cubebenol (100 or 250 μg/ml) for 4 h. Cell-free supernatants were collected, centrifuged, and measured for IL-1β, IL-6, and IFN-γ production by ELISA (BD Biosciences Pharmingen).

2.10. Statistical analyses

Survival data were analyzed using the log-rank test. All other data were evaluated using ANOVA or *t*-test. The Bonferroni test was used for post hoc comparisons, and statistical significance was set *a priori* at $P < 0.05$.

3. Results

3.1. α-Iso-cubebenol administration protects against sepsis-induced mortality

We first investigated whether α-iso-cubebenol, its chemical structure was shown in Fig. 1A, has anti-septic activity against polymicrobial sepsis using a CLP sepsis model. α-Iso-cubebenol administration strongly protected against mortality induced by CLP in a dose-dependent manner (Fig. 1B). Survival was strongly enhanced, reaching 80% when 15 mg/kg of α-iso-cubebenol was injected 2 h post-CLP and additionally three times at 12 h intervals (Fig. 1B). Inflammation of vital organs such as lung is associated with sepsis-induced mortality [1]. In this study, we also observed that CLP caused severe alveolar congestion and extensive thrombotic lesions in the lungs. Administration of α-iso-cubebenol markedly improved the pulmonary histopathology (Fig. 1C).

In the clinical setting, septic patients always receive antibiotics. The effect of α-iso-cubebenol treatment on survival in mice subjected to CLP in the presence of concomitant treatment with appropriate antibiotic regimen was tested. As shown in Fig. 1D, the administration of 10 mg/kg of both antibiotics (gentamycin plus cephalosporin) slightly increased the survival rate in the severe sepsis model. The administration of α-iso-cubebenol also slightly enhanced therapeutic effect against severe sepsis. Combination of α-iso-cubebenol with the antibiotics additionally increased survival rate (Fig. 1D).

3.2. α-Iso-cubebenol administration strongly enhances bacterial clearance

Since CLP surgery causes the release of intestinal contents including viable bacteria, CLP-induced lethality has been reported to be positively correlated with bacterial colony counts in the peripheral blood and peritoneal fluid [1]. Because α-iso-cubebenol administration markedly increased survival rate (Fig. 1), we examined the effects of α-iso-cubebenol on bacterial colony number in the CLP model. α-Iso-cubebenol administration strongly decreased bacterial numbers in the peritoneal fluid as well as peripheral blood (Fig. 2A and B). α-Iso-cubebenol administration significantly reduced the number of intraperitoneal bacteria by 80% (Fig. 2A), and almost completely eliminated intravascular bacteria (Fig. 2B). Since bactericidal activity is mediated by phagocytes which engulf bacteria [18], we also checked whether α-iso-cubebenol could affect phagocytic activity of mouse macrophages using fluorescein isothiocynate (FITC)-labeled dextran. Stimulation of Raw 264.7

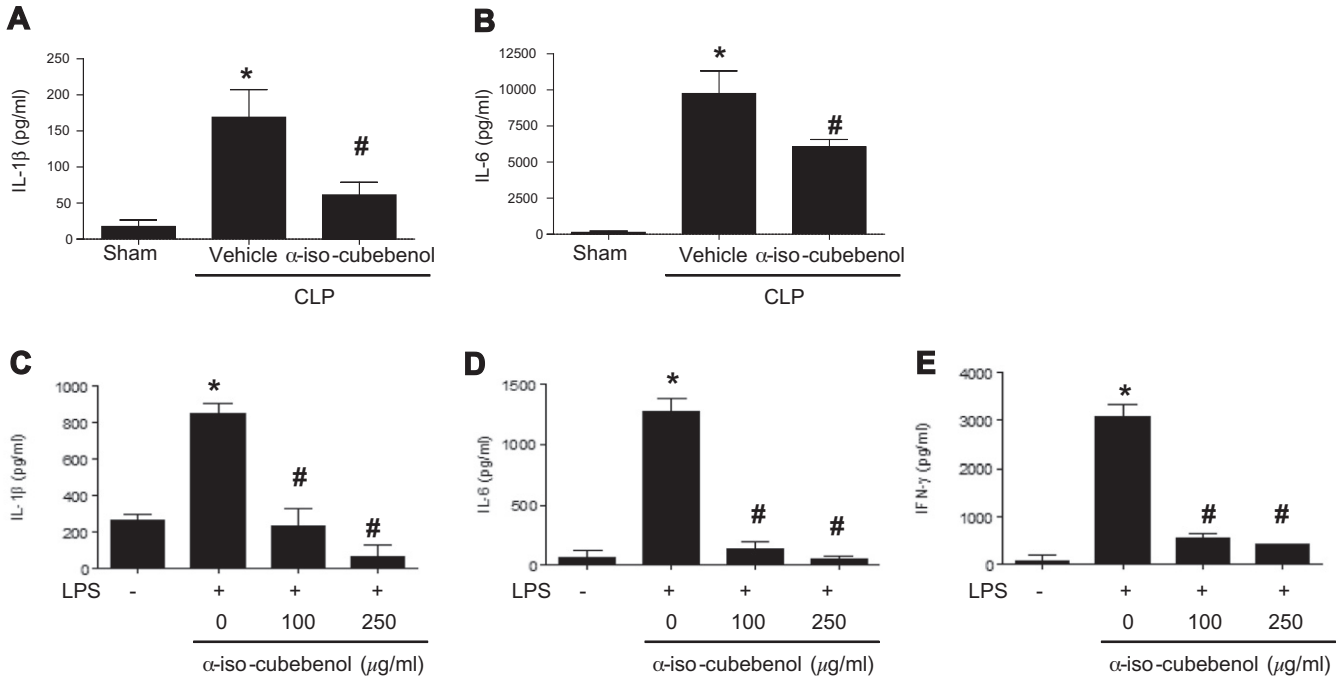

Fig. 4. Effects of α-iso-cubebenol on CLP- or LPS-induced cytokine production. (A–C) Vehicle (0.8% DMSO in PBS) or α-iso-cubebenol (15 mg/kg) was injected two times into CLP mice at 2 and 14 h post-CLP. Separate groups of animals were subjected to sham, CLP plus vehicle, or CLP plus α-iso-cubebenol treatment. Peritoneal fluids were collected at 24 h after CLP. Cytokine levels in the peritoneal fluid were determined by ELISA analysis. Panels A and B display results for IL-1β and IL-6, respectively. (C–E) Mouse splenocytes were stimulated with PBS or LPS (100 ng/ml). After 15 min, cells were subsequently stimulated with vehicle (0.1% DMSO in PBS) or α-iso-cubebenol (100 or 250 μg/ml) for 4 h. IL-1β (C), IL-6 (D), and IFN-γ (E) levels were measured by ELISA. Data are expressed as the mean ± SEM ($n = 8$). $^{*}P < 0.05$, compared with the value obtained from the sham (A and B) or not treated (C–E) control; $^{\#}P < 0.05$, significantly different from the CLP alone (A and B) or LPS alone (C–E) control.

cells with α-iso-cubebenol increased phagocytic activity (Fig. 2C). FITC-positive cells were increased from 45% to 72% by stimulation of the cells with α-iso-cubebenol (Fig. 2C). Bactericidal activity also has been demonstrated to be mediated by the phagocytic activity and reactive oxygen species generation of phagocytic cells such as neutrophils and macrophages [19]. α-Iso-cubebenol also strongly increased the production of superoxide anions in human neutrophils (Fig. 2D). These results suggest that α-iso-cubebenol stimulates bactericidal activity by enhancing phagocytic activity and superoxide anion production of phagocytes.

3.3. α-Iso-cubebenol administration inhibits CLP-induced lymphocyte apoptosis

Apoptosis of splenocytes and thymocytes is dramatically increased during the progression of sepsis. CLP treatment also increased the apoptosis of splenocytes and thymocytes, as detected by DNA fragmentation analysis (TUNEL assay) (Fig. 3A). α-Iso-cubebenol administration dramatically inhibited CLP-induced apoptosis of splenocytes and thymocytes (Fig. 3A). The numbers of apoptotic cells were quantified by counting TUNEL-positive cells (Fig. 3B). Apoptotic splenocytes and thymocytes in the α-iso-cubebenol administrated groups decreased to almost the level of sham control groups (Fig. 3B). Caspase-3 mediates the apoptosis of splenocytes and thymocytes in CLP sepsis [20]. Consistently, α-iso-cubebenol administration almost completely inhibited CLP-induced caspase-3 activity in splenocytes and thymocytes (Fig. 3C).

3.4. α-Iso-cubebenol inhibits proinflammatory cytokine production in vivo and in vitro

Pathogenesis of sepsis is associated with high levels of proinflammatory cytokines such as IL-1β and IL-6 [1]. To test the effects of α-iso-cubebenol on CLP-induced cytokine production, we measured the levels of IL-1β and IL-6 in peritoneal fluid with or without α-iso-cubebenol administration 24 h after CLP. As shown in Fig. 4A and B, CLP induced a dramatic increase in the production of the proinflammatory cytokines IL-1β and IL-6 within 24 h. α-Iso-cubebenol administration significantly decreased IL-1β and IL-6 levels at 24 h after CLP (Fig. 4A and B).

Next, we also examined whether α-iso-cubebenol could act directly on leukocytes to inhibit LPS-stimulated proinflammatory cytokine production. As reported, stimulation of freshly isolated splenocytes with 100 ng/ml LPS (as a prototypical microbial signal) strongly increased the levels of proinflammatory cytokines such as IL-1β, IL-6, and IFN-γ (Fig. 4C–E). α-Iso-cubebenol (100 μg/ml or 250 μg/ml) significantly decreased LPS-stimulated cytokine levels in splenocytes (Fig. 4C–E).

4. Discussion

With improvements in hospital care and increased awareness of deadly diseases, mortality rates from sepsis have decreased in recent years [3,4]. However, 1 in 1200 Americans die of severe sepsis annually [3,4]. Even though 17% of in-hospital deaths in the US are caused by sepsis, drugs that combat sepsis without antibiotics are not clinically available. Xigris, which had been approved by the US FDA as a therapeutic agent against sepsis, was withdrawn from the market. Thus, researchers are searching for new targets and therapeutic molecules to treat sepsis. In this study, we demonstrate that the therapeutic administration of α-iso-cubebenol after induction of sepsis by CLP can effectively inhibit CLP-induced lethality in mice. α-Iso-cubebenol appears to exert its therapeutic activity against sepsis through multiple mechanisms: (1) increase in bactericidal activity, (2) inhibition of leukocyte apoptosis, and (3) inhibition of proinflammatory cytokine secretion.

Sepsis-induced mortality is closely associated with organ failure, which can be caused by lymphocyte apoptosis [1]. Here

we observed the anti-apoptotic effects of α-iso-cubebenol in CLP sepsis mice (Fig. 3A and B). Among many mediators involved in lymphocyte apoptosis, caspase-3 is regarded to be a key player [20]. α-Iso-cubebenol administration dramatically inhibited CLP-induced caspase-3 activation (Fig. 3C). Therefore, α-iso-cubebenol may contribute to increase the survival rate of CLP mice by blocking lymphocyte apoptosis via inhibition of caspase-3.

α-Iso-cubebenol administration affects the cytokine profile in CLP sepsis mice. The production of proinflammatory cytokines including IL-1β and IL-6 is inhibited by α-iso-cubebenol (Fig. 4A and B), and LPS-induced production of IL-1β, IL-6, and IFN-γ in splenocytes is also inhibited (Fig. 4C–E). Since TLR4 mediates LPS-stimulated cellular signaling which leads to the induction of many proinflammatory molecules [21], α-iso-cubebenol may act by blocking LPS-induced TLR4-mediated signaling. Previously, we demonstrated that α-iso-cubebenol inhibits the expression of LPS-induced nitric oxide synthase and cyclooxygenase-2 in the Raw 264.7 mouse macrophage cell line [12]. Mechanistically, α-iso-cubebenol strongly inhibited nuclear translocation of the NF-κB p65 subunit in response to LPS in macrophages [12]. Therefore, α-iso-cubebenol may inhibit the production of LPS-induced proinflammatory cytokines by inhibiting NF-κB activity downstream of TLR4.

In conclusion, a novel natural product isolated from the *S. chinensis* fruit, α-iso-cubebenol, shows therapeutic effects against sepsis by enhancing bactericidal activity, inhibiting lymphocyte apoptosis, and modulating cytokine profile. α-Iso-cubebenol and its unidentified target(s) may prove useful in the development of efficient therapeutic agents against polymicrobial sepsis.

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SL, YC, and YB have pending patent applications. The other authors have no financial conflicts of interest.

References

[1] J. Cohen, The immunopathogenesis of sepsis, Nature 420 (2002) 885–891.
[2] D.C. Angus, W.T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, M.R. Pinsky, Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care, Crit. Care Med. 29 (2001) 1303–1310.
[3] G. Kumar, N. Kumar, A. Taneja, T. Kaleekal, S. Tarima, E. McGinley, E. Jimenez, A. Mohan, R.A. Khan, J. Whittle, E. Jacobs, R. Nanchal, Milwaukee Initiative in Critical Care Outcomes Research Group of Investigators, Nationwide trends of severe sepsis in the 21st century (2000–2007), Chest 140 (2011) 1223–1231.
[4] M.J. Hall, S.N. Williams, C.J. Defrances, A. Golosinskiy, Inpatient care for septicemia or sepsis: a challenge for patients and hospitals, NCHS Data Brief (2011) 1–8.
[5] W.D. Döcke, F. Randow, U. Syrbe, D. Krausch, K. Asadullah, P. Reinke, et al., Monocyte deactivation in septic patients: restoration by IFN-gamma treatment, Nat. Med. 3 (1997) 678–681.
[6] B.J. Czermak, V. Sarma, C.L. Pierson, R.L. Warner, M. Huber-Lang, N.M. Bless, et al., Protective effects of C5a blockade in sepsis, Nat. Med. 5 (1999) 788–792.
[7] R.S. Hotchkiss, K.W. Tinsley, P.E. Swanson, R.E. Schmieg Jr., J.J. Hui, K.C. Chang, et al., Sepsis-induced apoptosis causes progressive profound depletion of B and $CD4^+$ T lymphocytes in humans, J. Immunol. 166 (2001) 6952–6963.
[8] S.D. Wang, K.J. Huang, Y.S. Lin, H.Y. Lei, Sepsis-induced apoptosis of the thymocytes in mice, J. Immunol. 152 (1994) 5014–5021.
[9] C.A. Gogos, E. Drosou, H.P. Bassaris, A. Skoutelis, Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options, J. Infect. Dis. 181 (2000) 176–180.
[10] K. Reinhart, W. Karzai, Anti-tumor necrosis factor therapy in sepsis: update on clinical trials and lessons learned, Crit. Care Med. 29 (2001) S121–S125.
[11] L. Ulloa, K.J. Tracey, The "cytokine profile": a code for sepsis, Trends Mol. Med. 11 (2005) 56–63.
[12] Y.J. Lee, S.Y. Park, S.G. Kim, D.J. Park, J.S. Kang, S.J. Lee, S. Yoon, Y.H. Kim, Y.S. Bae, Y.W. Choi, Identification of a novel compound that inhibits iNOS and COX-2 expression in LPS-stimulated macrophages from *Schisandra chinensis*, Biochem. Biophys. Res. Commun. 391 (2010) 1687–1692.
[13] S.Y. Park, D.J. Park, Y.H. Kim, Y. Kim, Y.W. Choi, S.J. Lee, *Schisandra chinensis* α-iso-cubebenol induces heme oxygenase-1 expression through PI3K/Akt and Nrf2 signaling and has anti-inflammatory activity in *Porphyromonas gingivalis* lipopolysaccharide-stimulated macrophages, Int. Immunopharmacol. 11 (2011) 1907–1915.
[14] Y.W. Choi, H.J. Kim, S.S. Park, J.H. Chung, H.W. Lee, S.O. Oh, B.S. Kim, J.B. Kim, H.Y. Chung, B.P. Yu, C.D. Kim, S. Yoon, Inhibition of endothelial cell adhesion by the new anti-inflammatory agent alpha-iso-cubebene, Vascul. Pharmacol. 51 (2009) 215–224.
[15] S.D. Kim, H.Y. Lee, J.W. Shim, H.J. Kim, Y.H. Yoo, J.S. Park, et al., Activation of CXCR2 by extracellular matrix degradation product acetylated Pro-Gly-Pro has therapeutic effects against sepsis, Am. J. Respir. Crit. Care Med. 184 (2011) 243–251.
[16] H.Y. Lee, S.H. Jo, C. Lee, S.H. Baek, Y.S. Bae, Differential production of leukotriene B4 or prostaglandin E2 by WKYMVm or serum amyloid A via formyl peptide receptor-like 1, Biochem. Pharmacol. 72 (2006) 860–868.
[17] J. Wang, J.P. Silva, C.M. Gustafsson, P. Rustin, N.G. Larsson, Increased *in vivo* apoptosis in cells lacking mitochondrial DNA gene expression, Proc. Natl. Acad. Sci. USA 98 (2001) 4038–4043.
[18] J.C. Simard, M.M. Simon, P.A. Tessier, D. Girard, Damage-associated molecular pattern S100A9 increases bactericidal activity of human neutrophils by enhancing phagocytosis, J. Immunol. 186 (2011) 3622–3631.
[19] C. Erridge, Endogenous ligands of TLR2 and TLR4: agonists or assistants?, J Leukoc. Biol. 87 (2010) 989–999.
[20] R.F. Guo, M. Huber-Lang, X. Wang, V. Sarma, V.A. Padgaonkar, R.A. Craig, N.C. Riedemann, S.D. McClintock, T. Hlaing, M.M. Shi, P.A. Ward, Protective effects of anti-C5a in sepsis-induced thymocyte apoptosis, J. Clin. Invest. 106 (2000) 1271–1280.
[21] R. Salomao, M.K. Brunialti, M.M. Rapozo, G.L. Baggio-Zappia, C. Galanos, M. Freudenberg, Bacterial sensing, cell signaling, and modulation of the immune response during sepsis, Shock 38 (2012) 227–242.