BIOCHEMICAL PHARMACOLOGY 75 (2008) 914–922

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Baicalin inhibits macrophage activation by lipopolysaccharide and protects mice from endotoxin shock

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ARTICLE INFO

Article history:
Received 18 April 2007
Accepted 9 October 2007

Keywords:
Baicalin
Macrophage
Lipopolysaccharide
Nitric oxide
Endotoxin shock

ABSTRACT

Baicalin (BA) exhibits anti-inflammatory effect *in vivo* and *in vitro* and is used to treat inflammatory diseases. Here, we report that BA inhibits the activation of macrophage and protects mice from macrophage-mediated endotoxin shock. The experiments *in vitro* showed BA suppressed the increased generation of nitric oxide (NO) and expression of inducible nitric oxide synthase (iNOS) induced by LPS or Interferon-γ (IFN-γ) without directly affecting iNOS activity in RAW264.7 cells and peritoneal macrophages. Similarly, BA inhibited the production of reactive oxidative species (ROS), whereas augmented the level of intracellular superoxide dismutase (SOD). Moreover, BA inhibited the production of inflammatory mediators including tumor necrosis factor (TNF)-α, endothelin (ET)-1 and thromboxane A_2 (TXA_2) induced by lipopolysaccharide (LPS) in RAW264.7 cells. In animal model, BA protected mice from endotoxin shock induced by D-galactosamine (D-GalN)/LPS possibly through inhibiting the production of cytokine and NO. Collectively, BA inhibited the production of inflammatory mediators by macrophage and may be a potential target for treatment of macrophage-mediated diseases.

1. Introduction

Baicalin (BA, 7-glucuronic acid, 5,6-dihydroxyflavone) is a flavonoid compound purified from the medicinal plant *Scutellaria baicalensis* Georgi, which is used in the treatment of inflammatory diseases such as chronic hepatitis, bronchitis and atopic dermatitis [1–3]. BA and its metabolite, baicalein have been reported to have anti-inflammatory and anti-oxidative effect [4–6]. Previous studies show that BA inhibits exotoxin-stimulated T cell proliferation and suppresses the production of several inflammatory mediators by mononuclear cells [7]; BA binds to a variety of chemokines to limit their biological functions [8]; BA inhibits the activation of leukocyte through preventing leukocyte adhesion and down-regulating the generation of reactive oxygen intermediates [9]. Our previous study shows that BA suppresses the production of IFN-γ and TNF-α in primary culture splenocyte stimulated by Concanavalin A (Con A) [10]. Inflammatory mediator released from macrophage is vital to the regulation of immune response and the development of tissue injury. Thus, study of the effect of BA on macrophage activation is important for understanding its anti-inflammatory role. It has been reported that BA as well as structure analogical flavonoid baicalein and wogonin has inhibitory effect on iNOS expression in RAW264.7 cells [11]. However, whether BA inhibits the production of nitric oxide and other inflammatory mediator in primary

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.10.009

peritoneal macrophage remains largely unknown. Moreover, there was no experiment *in vivo* to support the beneficial effect of BA on macrophage-dependent diseases.

Macrophage can be activated by LPS to overproduce inflammation mediators including nitric oxide (NO), TNF-α, ET-1, thromboxane A_2 (TXA_2) and ROS [12–14]. The expression of iNOS and the production of large quantities of NO stimulated by LPS or synergistically by LPS and other cytokines are proved to contribute to pathophysiology of endotoxin shock [12,15]. TNF-α is the principle mediator in response of LPS and has a central role in the mortality of endotoxin shock [16]. ET-1, the potent vascular smooth muscle constrictor, was synthesized and released primarily from monocyte other than endothelial cells during the endotoxin shock [14]. ET-1 triggers the release of other vasoconstrictive mediators such as thromoxane A_2 in the endotoxemia, which mediate the hyperresponsiveness of the portal circulation to ET-1. Prolonged and excessive rise of ET-1 and TXA_2 may lead to decreased organ perfusion and organ damage [17].

The objective of the present paper is to investigate whether BA affects the production of inflammatory mediators by macrophage to inhibit inflammation. Our results suggest that BA suppresses the production of inflammatory factors in primary cultured peritoneal macrophage as well as RAW264.7 cells and protects mice against D-GalN/LPS-induced endotoxin shock.

2. Methods

2.1. Materials

Dulbcco's Modified Eagle's Medium (DMEM), penicillin and streptomycin was purchased from Invitrogen (Carlsbad, USA), LPS, D-GalN, cyclheximide from Sigma (St. Louis, MO, USA), Brewer's thioglycollate from Difco (Detroit, MI), baicalin (HPLC Content >98.0%) from Chongqing Green Valley Bio-tech Co. LTD. (Chongqing, China), recombinant mouse IFN-γ from BD PharMingen (San Diego, CA, USA), antibodies against iNOS from Chemicon International (Temecula, Canada). Other chemicals were of analytical grade.

2.2. Cell culture and treatment

Mouse peritoneal macrophages were elicited by i.p. injection of 1 ml of 4% Brewer's thioglycollate medium (Difco, Detroit, MI) into male Balb/c mice. Four days later, adherent peritoneal exudates cells were obtained by peritoneal lavage using ice-cold phosphate-buffered saline, seeded in dishes, and collected by removing the nonadherent cells after 2 h incubation at 37 °C. The RAW264.7, a mouse macrophage cell line, obtained from the American Type Culture Collection, was cultured in DMEM supplemented with 10%FBS, 100 mg/l penicillin and 70 mg/l streptomycin at 37 °C in a humidified incubator with 5% CO_2. Before experiment, all cells at a density of 5×10^5 cells/ml were cultured in non-serum medium for 20 h. The cell viability was assayed in 96-well plates. After overnight growth, the cells were treated with indicated concentrations of BA for 24 h with or without LPS (1 μg/ml) and the cell viability was determined using a Cell Counting Kit-8 (Dojindo Laboratories, Japan).

2.3. Measurement of TNF-α, ET-1, TXA_2 and nitride accumulation

The RAW264.7 cells or peritoneal macrophages in 96-well plates were treated with various concentration of BA for 1 h, and then treated with LPS (1 μg/ml) or recombinant mouse IFN-γ (100 U/ml) in the presence or absence of BA for 6 h (for TNF-α assay), 12 h (for ET-1 assay) or for 20 h (for NO assay). The concentration of TNF-α in the supernatant was determined by the ELISA Kit (Biosource International, USA). The production of TXA_2 was estimated by measuring the levels of TXB_2 (a relatively stable prostanoid derived from TXA_2) in the medium. The ET-1 and TXB_2 were assayed by radioimmunoassay kits (Beijing Chemclin Biotech Co. Ltd., China) and nitric oxide was assayed by nitric oxide colorimetric assay kit (Nanjing Jiancheng Biotechnology Institute, China) according to manufactures' protocol.

2.4. Measurement of iNOS enzyme activity

RAW264.7 cells were stimulated with 100 U/ml IFN-γ or 1 μg/ml LPS for 24 h to induce the iNOS protein. Then cells were depleted the stimuli and treated with 1 μg/ml cycloheximide (a translational inhibitor) to block further induction of iNOS protein. At the same time, various concentration of BA was added in the medium. After incubation for another 24 h, the supernatant were collected to measure the nitride accumulation. The iNOS activity was indirectly reflected by the nitride concentration.

2.5. Measurement of intracellular ROS and SOD

Dichlorofluorescin diacetate (DCFH-DA) was used as a substrate for measuring intracellular oxidant production. DCFH-DA is a stable, nonfluorescent and nonpolar compound that can diffuse through cell membranes. Once inside the cell, the acetyl groups are cleaved by cytosolic enzymes to form the polar nonfluorescent dichlorofluorescein (DCFH), and then rapidly oxidized to highly fluorescent dichlorofluorescein (DCF) in the presence of ROS. Cells were incubated with BA for 30 min and then incubated with LPS (1 μg/ml) and DCFH-DA (5 μM) at 37 °C for 30 and 60 min. Then ROS generation was measured by fluorimeter (BMG, NOVOstar Germany) with excitation at 485 nm and emission at 520 nm. For measurement of SOD, the cells were pretreated with BA for 1 h, and then some of cells were harvested, the other cells were treated with LPS for 20 h and harvested. The cells were crashed using the freeze-thaw method and sonication (60 W with 0.5 s interval for three times). The cell lysate was centrifuged at $10{,}000 \times g$ for 15 min and the supernatant was used to measure the cellular SOD with WST-1 based SOD inhibition assay (Dojindo Laboratory, Japan).

2.6. Animal experiments

The male Balb/c mice weighting 18–22 g were obtained from the Shanghai Laboratory Animal Center; the animals were

acclimated in animal rooms for 3 days. Ten mice were used for each group. In BA treated groups, mice were intraperitoneally given BA (100 mg/kg, dissolved in 0.85% NaCl, adjust to pH 7.4) three times within 24 h. The mice in normal group and LPS/D-GalN group received an equivalent volume of vehicle. To determine the survival rate, lethal dose of LPS (20 μg/kg) and D-GalN (700 mg/kg) were used at 1 h after last injection of BA or vehicle and the mice were observed for 48 h. To investigate the protective effect of BA on organ injury, non-lethal dose of LPS (15 μg/kg) and D-GalN (300 mg/kg) was used. The mice were sacrificed by decapitation at indicated time; blood and liver were collected for further examinations. All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica (Shanghai People's Republic of China). Serum ALT, AST and total bilirubin were analyzed according to the standard procedures. Serum TNF-α was determined using the ELISA Kit (Biosource International, Camarillo, California, USA). Serum NO was determined as mentioned above. Liver tissue was stained with hematoxylin-eosin (H&E). Apoptosis were analyzed with *in situ* cell Death Detection Kit (Roche, Basel, Switzerland).

2.7. Western blot analysis

The lysates (20 μg) were separated by SDS 8%-Polyacrylamide gel electrophoresis and transferred to polyvinylidene difluroide (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% skim milk for 1 h and incubated with the first antibodies; iNOS (1:5000) or GAPDH (1:1000). Immunoreactive bands were then detected by incubation with horseradish peroxidase conjugated anti-rabbit or anti-mouse IgG and visualized with the ECL reagents (Amersham, Piscataway, NJ, USA).

2.8. Statistical methods

All results were expressed as mean ± S.D. After homogenetic analysis, homogeneous data were analyzed with one-way analysis of variance (one-way ANOVA) and a post hoc test of least significant difference (LSD). To determine inter-group differences, heterogeneous data were analyzed using the independent sample *t*-test. Difference in survival rate was identified by log-rank test. A value of $P < 0.05$ was considered significance, $P < 0.01$ as highly significance.

3. Results

3.1. BA inhibited the increase of cell viability

Cell viability was examined to investigate the potential cytotoxicity of BA on RAW264.7 macrophages and peritoneal macrophages. The cell viability at different concentrations of BA was >90% in RAW264.7 cells and peritoneal macrophages (data not shown). These results showed BA had a low toxicity in RAW264.7 macrophages and peritoneal macrophages. Therefore, in the following experiment, the effect of BA (even at the concentration of 100 μg/ml) on the production of inflammatory mediators was not the result of a possible cytotoxic effect on these cells. LPS treatment augmented the cell viability in RAW264.7 cells; co-incubation with BA (0.1–50 μg/ml) reduced the augmentation of cell viability induced by LPS as shown in Fig. 1. In peritoneal macrophages, LPS treatment alone or co-incubation with BA did not affect the cell viability (data not shown).

3.2. BA inhibited the production of NO through down-regulating the expression of iNOS instead of affecting the enzyme activity

NO production in RAW264.7 cells and peritoneal macrophages was investigated by measuring the nitrite accumulation in the culture media. BA did not affect the basal production of NO (data not shown). After treatment with LPS (1 μg/ml) or IFN-γ (100 U/ml) for 20 h, nitrite concentration in the media increased dramatically by about 10-fold in RAW264.7 cells and by about 3.5-fold in peritoneal macrophages. Significant dose-dependent inhibition of nitrite accumulation was detected when RAW264.7 cells were incubated with different concentrations of BA together with LPS or IFN-γ (Fig. 2A). The same results were also found in peritoneal macrophages except that low dose of BA (<1 μg/ml) slightly increased the production of NO (Fig. 2B). To investigate whether the inhibition of NO production is due to the reduction of iNOS expression or the affection of iNOS enzyme activity, we assessed the effect of BA on iNOS expression or on iNOS enzyme activity. Western blot results showed that normal RAW264.7 cells did not express detectable iNOS protein, treatment with LPS or IFN-γ for 6 h drastically increased iNOS protein expression. Co-treatment with BA significantly inhibited the expression of iNOS protein in a dose-dependent manner (Fig. 2C). The same results were also found in peritoneal macrophages (Fig. 2D). However, BA failed to affect iNOS enzyme activity, since the nitrite levels remained

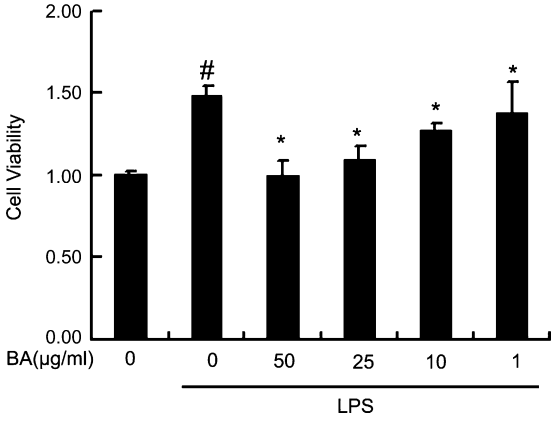

Fig. 1 – Effect of BA on increased cell viability stimulated by LPS in RAW264.7 cells. RAW264.7 cells were incubated with LPS (1 μg/ml) in the presence or absence of BA for 24 h. Normal cell viability was set to 1.0 and the change of cell viability in LPS-stimulated cells was shown as fold of that. Each column shows the mean ± S.D. of triplicate respective determinations. #P < 0.01 vs. control cells; *P < 0.01 vs. LPS-treated cells.

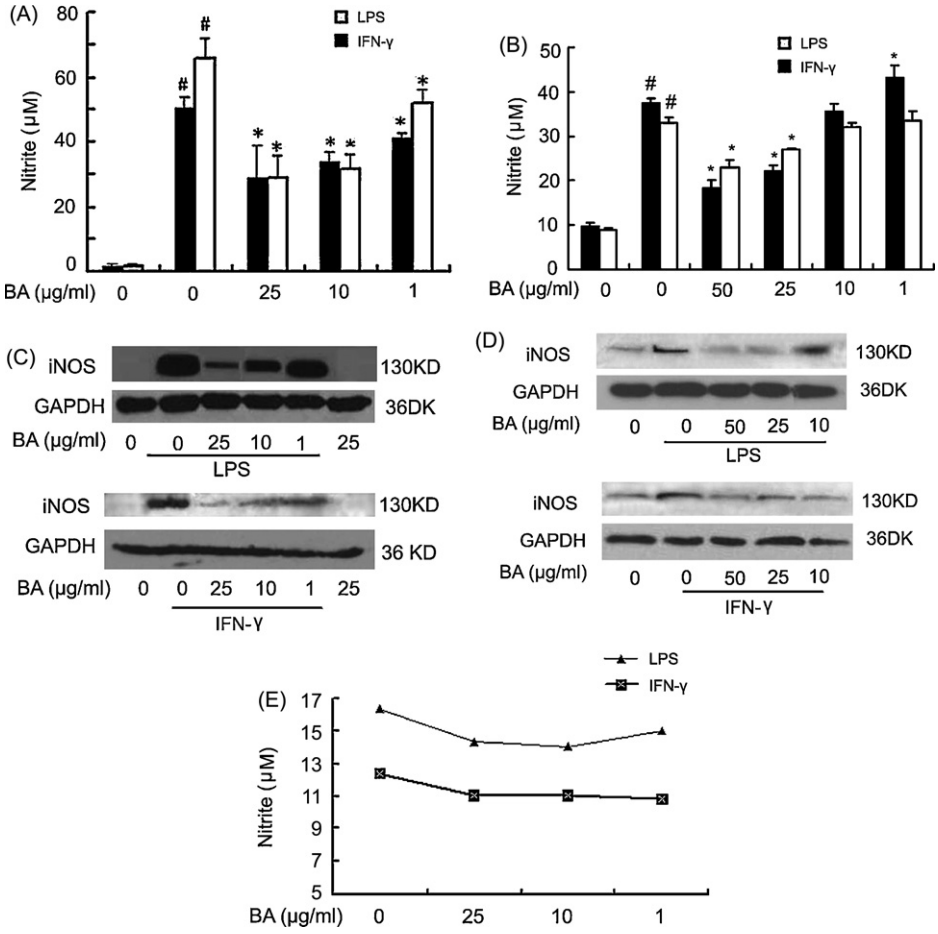

Fig. 2 – Effect of BA on the production of nitride oxide and expression of iNOS in LPS-or IFN-stimulated RAW264.7 cells and peritoneal macrophages. RAW264.7 cells or peritoneal macrophages were incubated with the indicated concentrations of BA for 1 h before being incubated with LPS (1 μg/ml) or IFN-γ (100 U/ml) for 20 h (for NO assay) or for 6 h (for iNOS assay) (A and B), nitrite accumulation in RAW264.7 cell (C) or peritoneal macrophage (D); $^{\#}P < 0.01$ vs. control cells; $^{*}P < 0.01$ vs. LPS or IFN-γ-treated cells. (C and D) iNOS expression in RAW264.7 cell (C) or peritoneal macrophage (D); iNOS expression was analyzed using the whole-cell lysate by Western blot. This is a representative blot of three separate studies. (E) iNOS enzyme activity. Each column shows the mean ± S.D. of triplicate respective determinations.

unchanged in cells treated with or without BA in the iNOS enzyme activity experiments (Fig. 2E).

3.3. *BA inhibited intracellular ROS stimulated by LPS whereas augmented intracellular SOD level in RAW264.7 cells*

Oxidative stress is important for the activation of macrophage and SOD has a central role in the defense against oxidative stress [18]. Intracellular ROS produced very quickly after LPS stimulation (within 30 min). BA significantly suppressed the production of intracellular ROS at all the test concentrations in RAW264.7 cells (Fig. 3A). Similarly, BA also significantly suppressed ROS in peritoneal macrophages (Fig. 3B). Further, BA-treated RAW264.7cells exhibited significantly higher levels of SOD compared with normal cells (Fig. 3C). At 20 h after LPS treatment, intracellular SOD increased slightly probably due to counterbalance of redox state. Compared with that, BA pretreated cells showed more augmentation of SOD (Fig. 3D).

3.4. *BA inhibited the production of inflammatory mediators induced by LPS in RAW264.7 cells*

Unstimulated RAW264.7 cells produced a low background level of TNF-α, ET-1 and TXB_2. Stimulation with LPS significantly increased the TNF-α, ET-1 and TXB_2 level by about 34-, 30- and 4-fold in the culture medium at 6 or 24 h, and these increases were effectively inhibited by pretreatment with BA in a dose-dependent manner (Fig. 4A–C).

3.5. *BA alleviated LPS/D-GalN induced endotoxin shock*

We next determined whether BA suppresses inflammatory response in mice. After lethal dose of LPS/D-GalN challenge, death of mice occurred at 6 h and 3 of 10 mice in D-GalN/LPS group died within 8 h, all mice in LPS-GalN group died within 24 h. No death occurred in the mice those were pretreated with BA (100 mg/kg) within 8 h and 3 of the 10 mice survived for 48 h (Fig. 5). The results showed BA prolonged the survival time and

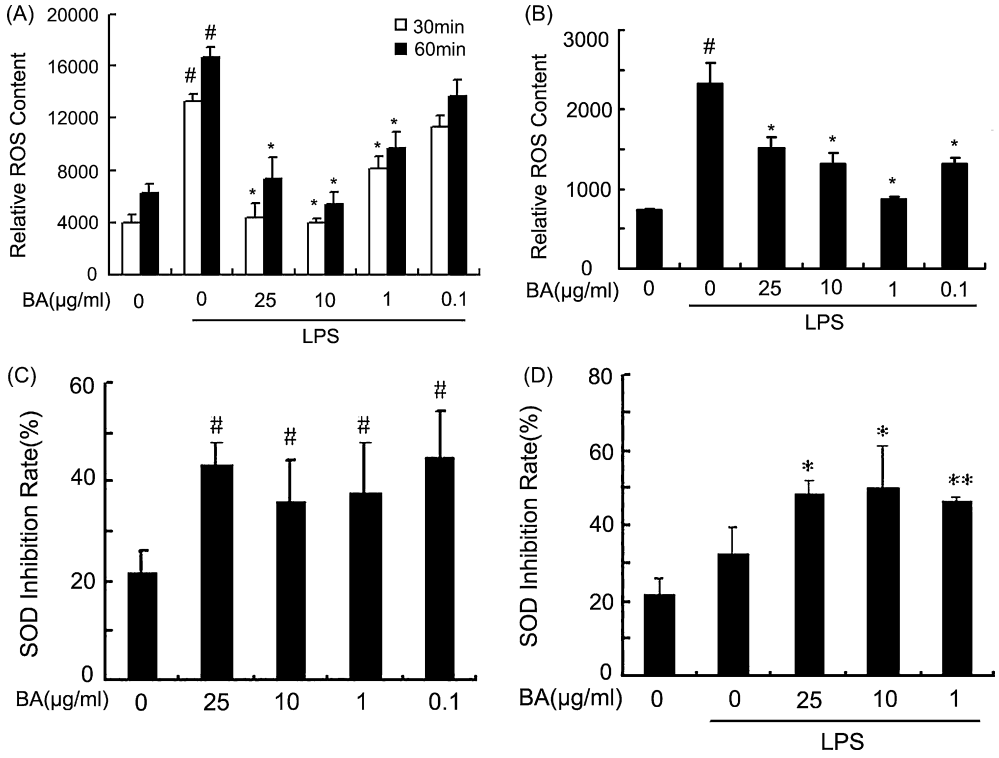

Fig. 3 – Effect of BA on ROS generation and cellular SOD level in LPS-stimulated RAW264.7 cells. (A and B) The production of ROS in RAW264.7 cell (A) or peritoneal macrophage (B). The cells were incubated with indicated concentrations of BA for 1 h before being incubated with LPS and DCFH-DA for 30 or 60 min for measure the production of ROS. (C and D) SOD activity. RAW264.7 cells were incubated with indicated concentrations of BA for 1 h, and then some cells were collected and others were treated with LPS for 20 h and then collected for SOD assay. Each column shows the mean ± S.D. of triplicate respective determinations. $^{\#}P < 0.01$ vs. control cells; $^{*}P < 0.01$ vs. LPS-treated cells; $^{**}P < 0.05$ vs.; LPS-treated cells.

increased the survival rate. The degree of liver injury was judged by elevated ALT, AST, total bilirubin and pathological change. Serum ALT, AST and total bilirubin were significantly increased by 16-, 6- and 12-fold over those in normal mice at 8 h after D-GalN/LPS challenge. Pretreatment with BA significantly reduced the elevation of serum ALT, AST and total bilirubin by 50, 60 and 80%, suggesting the marked protective effect against LPS/D-GalN induced liver injury (Table 1). In the D-GalN/LPS-treated group, broad hemorrhagic necrosis and apoptotic positive hepatocytes were found at 8 h after endotoxin challenge. In the BA-pretreatment group, only slight hemorrhage and degeneration were observed and apoptotic cell death was significantly alleviated (Fig. 6). These results suggested that BA-pretreatment appeared to be effective for suppressing apoptotic cell death and alleviating the microcirculatory failure in the endotoxin shock.

3.6. *BA-pretreatment decreased the level of serum nitrite accumulation and TNF-α*

D-GalN/LPS treatment caused significant increase of serum TNF-α level and nitrite accumulation. BA-pretreated mice showed significantly lower TNF-α level and nitrite accumulation than D-GalN/LPS treated mice (Table 1).

4. Discussion

BA has been known to have a variety of beneficial effects including antimicrobial, anti-inflammatory activities [1,2,4–6]. In the present study, we reports BA suppresses the production of NO, ROS ET-1, TXA_2 and TNF-α, production in murine macrophages stimulated by LPS or IFN-γ *in vitro* and confers

Table 1 – Effect of baicalin on the serum animotransferases, total bilirubin, TNF-α and NO level in LPS/D-GalN induced liver injury

Treatment	ALT (IU/ml)	AST (IU/ml)	Bilirubin	TNF-α (μg/ml)	NO (μM)
Normal	29.40 ± 25.51	31.34 ± 11.89	0.55 ± 0.23	3.38 ± 1.89	15.43 ± 5.22
D-GalN/LPS treated	493.73 ± 48.84$^{\#}$	196.12 ± 24.45$^{\#}$	10.18 ± 2.34$^{\#}$	291.55 ± 138.67$^{\#}$	297.33 ± 30.81$^{\#}$
D-GalN/LPS + BA treated	240.35 ± 101.53*	79.15 ± 39.92*	2.3 ± 2.018*	76.11 ± 38.17*	218.72 ± 19.00*

($n = 8$).
$\#p < 0.01$ vs. normal group; $^{*}<0.01$ vs.D-GalN + LPS treated group.

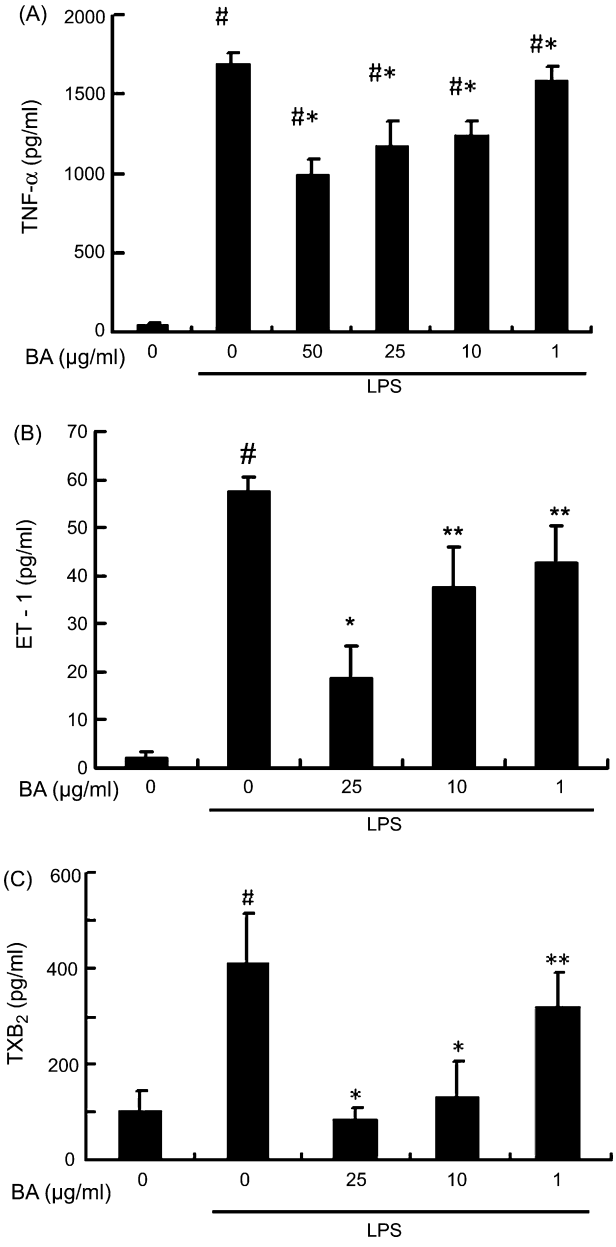

Fig. 4 – Effect of BA on the production of inflammatory mediators in LPS-stimulated RAW264.7 cells. RAW264.7 cells were incubated with the indicated concentrations of BA for 1 h before being incubated with LPS (1 μg/ml) or IFN-γ (100 U/ml) for 6 h (for TNF-α assay) or for 20 h (for ET-1, TXB_2 assay). (A) TNF-α; (B) ET-1; (C) TXB_2; $^{\#}P < 0.01$ vs. control cells; $^{*}P < 0.01$ vs. LPS-treated cells.

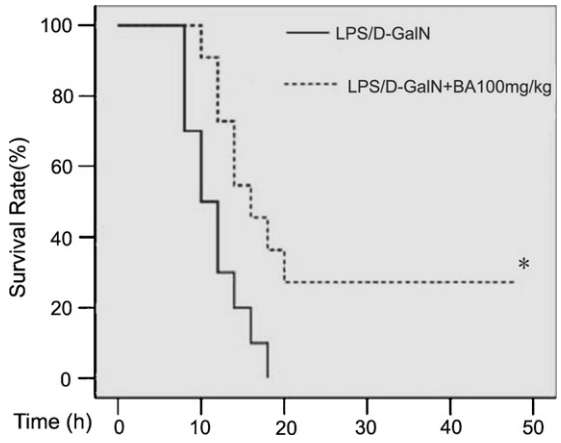

Fig. 5 – Effect of BA on the survival rate of mice challenged by D-GalN/LPS. 100 mg/kg BA or vehicle was intraperitoneally administrated three times before D-GalN/LPS challenge within 24 h. All Mice were intraperitoneally injected with lethal dose of D-GalN/LPS and observed for 48 h. Each group consisted of 10 mice. $^{*}P < 0.01$ vs. D-GalN/LPS-treated mice.

protection against D-GalN/LPS-induced lethal shock *in vivo*. Considering the important role of macrophage activation in the endotoxin shock [19,20], these findings suggest that BA exerts its anti-inflammatory effect mainly through suppressing the macrophage activation.

TNF-α and large amounts of NO produced by iNOS in macrophages are acknowledged to be importantly involved in tissue damage observed in endotoxin shock [21,22]. The present study showed BA suppressed the production of TNF-α and NO in murine macrophages stimulated by LPS or IFN-γ. Considering a critical role of NO and TNF-α in septic shock, increased survival of mice and reduction of liver damage might be mediated by inhibition the production of NO and TNF-α. Next, the present study showed that BA inhibited NO production through inhibiting the induction of iNOS expression instead of affecting the iNOS enzyme activity. It seemed that BA had two-way regulation of NO production in peritoneal macrophages *in vitro*. Relatively high dose of BA showed inhibitory effect of NO production, while low dose of BA showed slightly promotional effect. Further study would focus on whether these two-way regulations also existed *in vivo*. It is well known that iNOS can be synergistically induced by LPS, IFN-γ or TNF-α in the inflammatory process [23,24]. LPS or TNF-α provides the second signal which mediates the induction of iNOS expression in response to IFN-γ [25]. Thus, the reduction of TNF-α by BA would possibly abrogate the further autocrine induction of iNOS and contribute to reduction of NO generation.

It is well known that excessive NO and ROS production in the same location may lead to especially detrimental consequences because NO reacts with ROS to form RNS, a powerful oxidant to cause local tissue injury [26,27]. In the endotoxin shock, this reaction may be in association with development of hepatic injury around blood vessels [28]. BA was proved to scavenge ROS as well as RNS in endothelial cell and protect endothelial cell injury [29]. The research in our lab showed that BA had the protective effect on hepatocyte injury induced by peroxynitrite (data not published). The present study showed that BA suppressed the production of ROS and NO in RAW264.7cell as well as peritoneal macrophage. Moreover, our results suggested except scavenging free radicals effect, BA also augmented SOD, which is known to protect ROS and RNS induced tissue injury, to provide sufficient and persistent anti-oxidant efficacy. All these

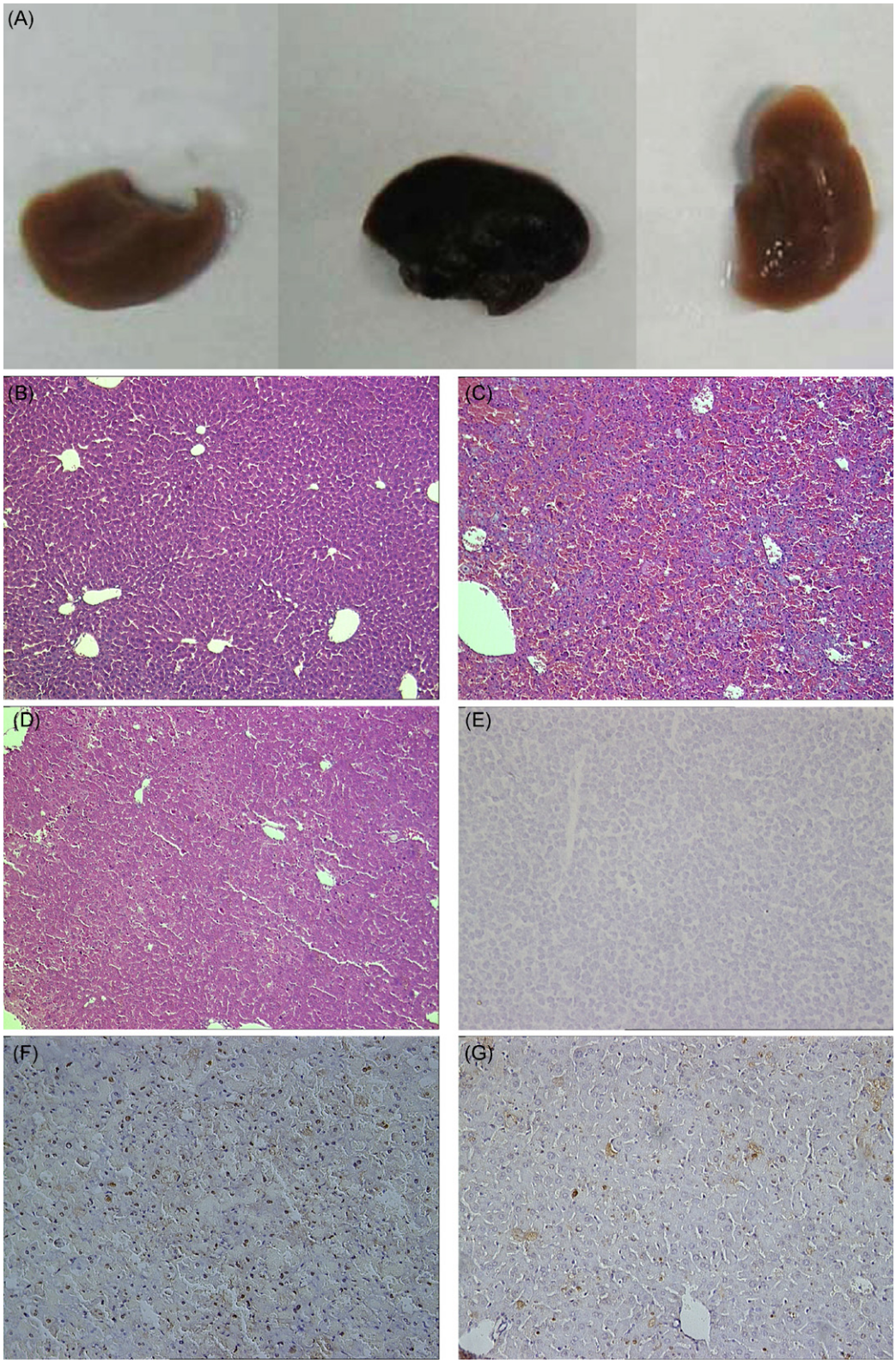

Fig. 6 – Effect of BA on the histological change of liver induced by D-GalN/LPS. (A) Gross liver appearance; the left one is normal mouse liver, the middle one is D-GalN/LPS challenged mice liver, the right one is D-GalN/LPS + BA (100 mg/kg) treated mice liver; (B–D) H&E staining; (E–G) Apo-BrdU *in situ* DNA fragmentation staining. (B and E) normal mice; (C and F) D-GalN/LPS challenged mice; (D and G) D-GalN/LPS + BA (100 mg/kg) mice. Note the remarkable differences of gross liver appearance between D-GalN/LPS treated group and D-GalN/LPS + BA (100 mg/kg) treated mice treated group. In Apo-BrdU *in situ* DNA fragmentation staining, the number of apoptotic cells was significantly low in mice pretreated with BA. Each group consisted of 8 mice.

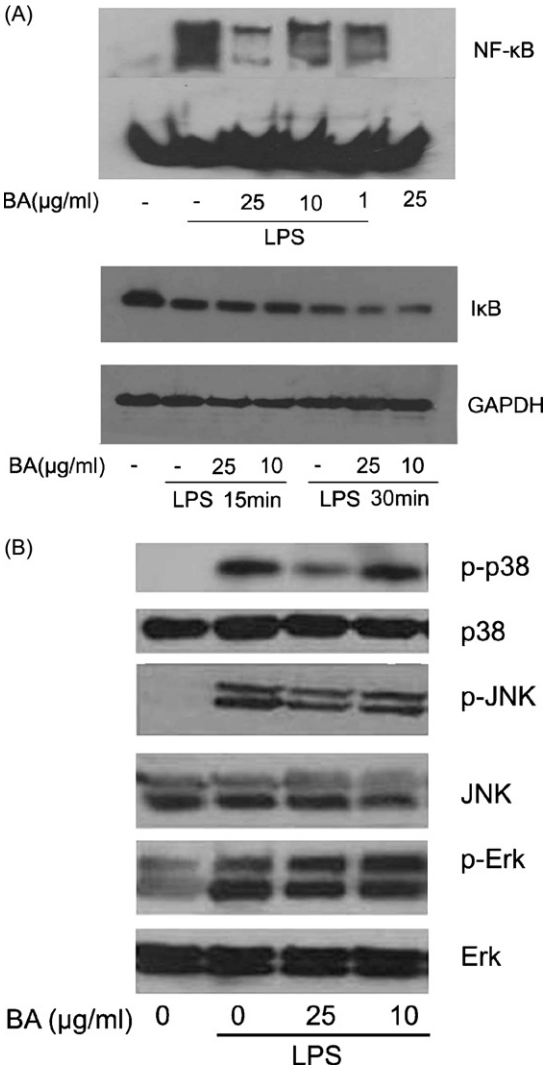

Fig. 7 – Effect of BA on LPS-induced phosphorylation of MAP kinases and NF-κB activation in RAW264.7 cells. RAW264.7 cells were treated with the vehicle or indicated concentrations of BA for 1 h before being incubated with LPS (1 μg/ml) for 30 min. The whole-cell lysates were analyzed by Western blot analysis and nuclear protein was isolated to perform EMSA. (A) MAP kinase activation; (B) NF-κB activation and I-κB degradation. This is a representative blot of three separate studies.

results provided the possible mechanisms whereby BA exerted a cytoprotective effect in endotoxin shock.

It is reported that the release of ET-1 and the followed induction of TXA_2 have important roles in the endotoxin shock. Macrophages rapidly and dramatically increase the production of ET-1 and TXA_2 when stimulated by LPS *in vitro* and Gram-negative bacteria *in vivo* [17,30]. Although it was theorized that the elevated levels of ET-1 initially encountered in sepsis may be attributed to the normal homeostatic response to hypotension, its prolonged and excessive rise may lead to excessive vasoconstriction in some vascular beds and result in the severely reduced sinusoidal blood flow as well as impaired oxygen delivery [31]. Thus, reduction of ET-1 and TXA_2 production in activated macrophage is at least partially response to the alleviated microcirculatory failure and less tissue damage caused by LPS/D-GalN in BA pretreated mice.

We next investigated whether BA suppressed the MAP kinase activation or NF-κB activity that regulated the expression of NO and other inflammatory mediator in LPS-stimulated macrophage. The results showed that relatively high dose of BA can inhibit the activation of p38 and JNK in RAW264.7 (Fig. 7A). However, BA cannot inhibit the NF-κB activation and I-κB degradation (Fig. 7B). Inhibition of NF-IL-6/C-BEP-β activation was considered to be the major determinant for the suppression of iNOS and COX-2 by baicalein (which is the metabolite of baicalin) in microglia and RAW264.7 cells. However, BA itself had no effect on NF-IL-6/C-BEP-β activation in macrophage or microglia [32,33].

In summary, the present study suggests that BA lowered NO and various inflammatory mediator production in murine macrophage stimulated by LPS or IFN-γ. In addition, this is the first *in vivo* experiment to demonstrate the beneficial effect of BA on endotoxin shock. Therefore, BA maybe used to treat inflammatory diseases in which hyperresponse of macrophage is the main cause.

Acknowledgments

We thank to Miss Hua Sheng, Miss Heng-lei Lu and Mrs Xing-Ju Yuan for their assistance in the experiments. We gratefully acknowledge Dr. Yi-zheng Wang for valuable comments and guidance.

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