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Short communication

# Berberine inhibits LPS-induced TF procoagulant activity and expression through NF-κB/p65, Akt and MAPK pathway in THP-1 cells

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ABSTRACT

*Background:* Considering the key role of TF in coagulation of sepsis or acute lung injury (ALI), we investigated whether berberine (BBR) could inhibit TF expression and procoagulant activity and explored its possible mechanism.
*Methods:* The effects of berberine on the expression, procoagulant activity of TF and related signal pathways induced by lipopolysaccharide (LPS) were observed in THP-1 cells.
*Results:* Our results showed that berberine could inhibit LPS-induced TF activity and expression, and down-regulate NF-κB, Akt and MAPK/JNK/p38/ERK pathways.
*Conclusion:* Berberine inhibits TF expression and related pathway, which provides some new insights on its mechanism for sepsis treatment.


## Introduction

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are characterized by pulmonary edema and neutrophilic inflammation and cause acute respiratory failure in patients of all ages. Recent research has shown that abnormalities in coagulation and fibrinolysis are present in ALI and may be the result of ongoing inflammation in the lungs. Thrombin formation and fibrin deposition, which appear as hyaline membranes lining the denuded alveolar surface, have become one of the typical pathological signs in ALI/ARDS patients [1,2]. Tissue factor (TF), a 47-kDa protein, is the only blood coagulation factor that exists outside the plasma in normal cells and tissue. As the initiator of the coagulation cascade reaction, TF participates in ALI/ARDS and locally activates various cytokines in the lungs as well as the systemic inflammatory reaction in patients suffering from ALI/ARDS [1]. TF-related signaling pathways, including the NF-kappa B and MAPK/JNK/p38/ERK pathways, are involved in ALI development after lipopolysaccharide (LPS) stimulation [2–4], which causes the production of intracellular reactive oxygen species through Toll-like receptor 4. Multiple cell types, including endothelial cells, alveolar macrophages and monocytes, have been associated with ALI [5,6]. Furthermore, monocytes synthesize TF in response to various pathophysiological procedures that are important in *in vitro* models of sepsis [7]. After stimulation with agents such as lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α) and phorbol ester (PMA), the procoagulant activity and expression of tissue factor are up-regulated in human monocytes, which results in inflammation and the development of severe acute coronary syndrome (ACS), sepsis or ALI [8]. Additionally, inhibitors against the complex of TF and FVII can balance coagulation and thrombus formation, resulting in fewer adverse effects such as bleeding compared with other anticoagulant drugs [9]. Blockade of the TF pathway may provide a specific therapeutic strategy for ALI or sepsis [1].

*Abbreviations:* ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BBR, berberine; DIC, disseminated intravascular coagulation; ERK, extracellular regulated protein kinases; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinases; NF-κB, nuclear factor (NF)-κB; PCA, procoagulant activity; TF, tissue factor.

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Berberine (BBR), an isoquinoline alkaloid, is the major active component of the Chinese medicinal herb *Rhizoma coptidis* (Huanglian) and has multiple biochemical and pharmacological activities, including lipid-lowering and anti-inflammatory effects [10,11]. Recent studies have shown that BBR possesses marked activities against sepsis and ALI [12–14]. Furthermore, we previously found that BBR has a significant inhibitory effect on the TF procoagulation activity (PCA) induced by LPS in human monocytes or THP-1 cells [15,16]. These findings indicate that inhibiting the TF pathway might be a possible action mechanism of BBR in the treatment of ALI; however, it is still unclear which signaling pathways are involved in this inhibition of TF.

Therefore, in the present study, we investigated the mechanism of BBR in down-regulating LPS-induced TF expression and activity in THP-1 cells to provide further support for its clinical use to treat sepsis and other diseases involving TF.

## Materials and methods

### *Materials*

The human monoblastic leukemia cell line THP-1 was obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Cell culture reagents were purchased from Gibco (Carlsbad, CA, USA) and PAA Laboratories GmbH. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum in a 95% air–5% $CO_2$ humidified atmosphere at 37 °C.

### *Chemicals and reagents*

Berberine chloride was obtained from the Nanjing Qingze Medical Technology Company (Nanjing, China). Curcumin chloride was kindly provided by Dr. Haixia Ge. LPS (from *Escherichia coli* O55:B5), the chromogenic substrate Xa and Triton X-100 were obtained from Sigma (St. Louis, MO, USA). The human prothrombin complex (300 IU, containing factor II, VII, IX and X) was obtained from Hualan Bioengineering Company (Xinxiang, China). The p65 inhibitor JSH-23, the Akt inhibitor LY294002, the JNK inhibitor SP600125 and the p38 inhibitor SB203580 were obtained from Alexis-Biochemicals (San Diego, CA, USA). Anti-TF antibody was purchased from R&D Systems (Minneapolis, MN, USA). Anti-p65 and anti-phospho-NF-κB/p65 antibodies, anti-JNK and anti-phospho-JNK antibodies, anti-p38MAPK and anti-phospho-p38MAPK antibodies, and anti-Akt and anti-phospho-Akt antibodies were purchased from Bioworld Technology (St. Louis Park, MN, USA). Anti-ERK antibodies and anti-phospho-ERK were purchased from Cell Signaling Technology (Beverly, MA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was obtained from Shanghai Kang Chen Bio-tech Inc. (Shanghai, China). The goat anti-rabbit IgG antibody was purchased from Wuhan Boster Biological Technology, LTD (Wuhan, China). All other reagents were of analytical grade.

### *Drug treatment*

THP-1 cells were grown in medium for 2 h before pretreatment with berberine at 0.01–1.0 μM or pathway inhibitors for 1 h and were stimulated with 500 ng/mL LPS for 5 h.

### *Simplified chromogenic assays for TF procoagulation activity*

Cells lysates were obtained by repetitive freezing and thawing and were incubated with 10 g/L human prothrombin complex in Tris–$CaCl_2$ buffer (pH 7.3) at 37 °C for 15 min. Thereafter, 0.5 mM chromogenic substrate Xa in Tris–EDTA buffer (pH 8.4) was added. The absorbance was read at 405 nm [16].

### *Western blotting analysis*

Protein expression was determined by western blotting analysis. The antibody against TF was used at a 1:800 dilution. Anti-p65 and anti-phospho-NF-kB/p65 antibodies, anti-JNK and anti-phospho-JNK antibodies, anti-p38MAPK and anti-phospho-p38MAPK antibodies, anti-Akt and anti-phospho-Akt antibodies, and anti-ERK antibodies and anti-phospho-ERK were used at 1:1000 dilutions. The blots were normalized to GAPDH expression (1:5000 dilution). The antigen–antibody complexes were then detected using a chemiluminescence system.

### *Immunofluorescence*

Treated cells were washed with cold PBS, fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. After blocking, the cells were incubated with the primary antibodies and FITC/TRITC-conjugated secondary antibodies. Fluorescence was detected by using an Axiovert 40 fluorescence microscope (Zeiss, Germany).

### *Statistical analysis*

The data were expressed as the means ± SEM and analyzed by one-way ANOVA or two-tailed unpaired Student's *t*-test. A probability value of less than 0.05 was considered statistically significant. All results are representative of at least three independent experiments.

## Results

### *Berberine attenuated LPS-induced TF-PCA and expression in THP-1 cells*

THP-1 cells were stimulated with LPS (500 ng/mL) for 5 h. As shown in Fig. 1A, berberine had an extremely significant suppressing effect on LPS-induced TF procoagulant activity at three concentrations between 0.01 and 1 μM. The inhibition rate of berberine on TF-PCA at the highest concentration of 1 μM was approximately 71%. Additionally, pretreatment with berberine (0.01–1 μM) inhibited LPS-induced TF protein expression (Fig. 1B). Similarly, curcumin inhibited LPS-induced TF expression and activity.

### *Involvement of signaling pathways in the inhibitory effects of berberine on TF procoagulant activity in THP-1 cells*

To investigate the mechanism of the inhibitory effect of berberine on TF-PCA in THP-1 cells, we investigated the effect of inhibitors of various signaling pathways on the inhibitory activity of berberine. Pretreatment with the p65 inhibitor JSH-23 (8 μM), the Akt inhibitor LY294002 (10 μM), the JNK inhibitor SP600125 (40 nM) and the p38 inhibitor SB203580 (70 nM) for 1 h significantly decreased the TF procoagulant activity of the cells, as the relative inhibition rates were approximately 31.8%, 40.7%, 16.9% and 53.7%, respectively (Fig. 2). When a combination of the four pathway inhibitors was used with berberine, the inhibiting effect of berberine on TF-PCA was decreased by varying degrees, indicating that the NF-kappa B, Akt and MAPK signaling pathways all participate in the inhibitory effect of berberine on the TF-PCA.

### *Effects of berberine on the LPS-induced activation of p65, Akt, JNK, p38 and ERK in THP-1 cells by Western blotting assay*

To further determine the mechanism by which berberine inhibits TF expression and TF activity, we investigated the expression and phosphorylation of p65, Akt, JNK, ERK and p38