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Glycyrrhizic acid pretreatment prevents sepsis-induced acute kidney injury via suppressing inflammation, apoptosis and oxidative stress

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ABSTRACT

Glycyrrhizic acid (GA), an active ingredient in licorice, has multiple pharmacological activities. The aim of our study was to investigate the molecular mechanism involved in the protective effects of GA in lipopolysaccharide (LPS) stimulated rat mesangial cells (HBZY-1) and septic rats. Sepsis model was established by injection of 5 mg/kg LPS in rats or incubation with 1 μg/ml LPS for 24 h in HBZY-1 cells. A variety of molecular biological experiments were carried out to assess the effects of GA on inflammation, apoptosis, and oxidative stress. First we found that GA alleviated sepsis-induced kidney injury *in vivo*. Furthermore, GA suppressed inflammatory response *in vivo* and *in vitro*. Additionally, GA inhibited cell apoptosis and the changes in expressions of apoptosis related proteins induced by LPS. Moreover, GA markedly inhibited oxidative stress induced by LPS via activation of ERK signaling pathway. Finally GA could inhibit the activation of NF-κ B induced by LPS. Our present study indicates that GA has a protective effect against sepsis-induced inflammatory response, apoptosis, and oxidative stress damage, which provides a molecular basis for a new medical treatment of septic acute kidney injury.

1. Introduction

Sepsis is a systemic inflammatory response syndrome caused by infection (Fry, 2012), which, when excessive, may progress to organ failure and death (Hotchkiss et al., 2013). Although people have made continuous improvement in the clinical technology for intensive care, the latest epidemiological studies show that each year more than 18 million people are infected by severe sepsis in the world and the number is rising at an annual rate of 1.5–8.0%. In addition, sepsis is a serious disease and has high mortality rates of up to 60%. About 14,000 people die from sepsis complications in the world everyday (Dombrovskiy et al., 2007). Thus, the identification of new therapeutic and preventive approaches is crucial.

GA is one of the active ingredients in licorice, which has strong effects on increasing the body's immune function and inhibiting the proliferation of various cancer cells. Especially for human immunodeficiency virus (HIV) the inhibition rate of GA was more than 90% (Ming and Yin, 2013; Ploeger et al., 2001). Recent studies also have shown that GA has a variety protective effects on lung. For example, GA could alleviate the lung injury induced by benzopyrene (Ma et al., 2013). In addition, GA also has the therapeutical effect on asthma by regulating the immune function (Qamar et al., 2012). The study by Ni et al. has also showed that glycyrrhizin treatment is associated with attenuation of lipopolysaccharide-induced acute lung injury by inhibiting cyclooxygenase-2 and inducible nitric oxide synthase expression (Ni et al., 2011). Furthermore, GA can inhibit the expressions of inflammatory cytokines in macrophages induced by LPS (Wang et al., 2013). However, the effect of GA on sepsis-induced kidney injury and its related molecular mechanism is poorly understood.

In this study, we investigated the protective effect of GA against inflammatory response, apoptosis, and oxidative stress in LPS-stimulated HBZY-1 cells and septic rats.

2. Materials and methods

2.1. Cell line and culture

HBZY-1 cells were obtained from China Center for Type Culture Collection and cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (Hyclone, USA), 100 μg/ml streptomycin and 100 U/ml penicillin (Hyclone, USA), at 37 °C, under a 5.0% CO_2 atmosphere.

2.2. Drugs and antibodies

GA was purchased from Meilun Biotec Co., Ltd (Dalian, China). GA was dissolved in DMSO as a stock concentration of 180 mM and stored in the dark at −20 °C. The chemical structural of GA is

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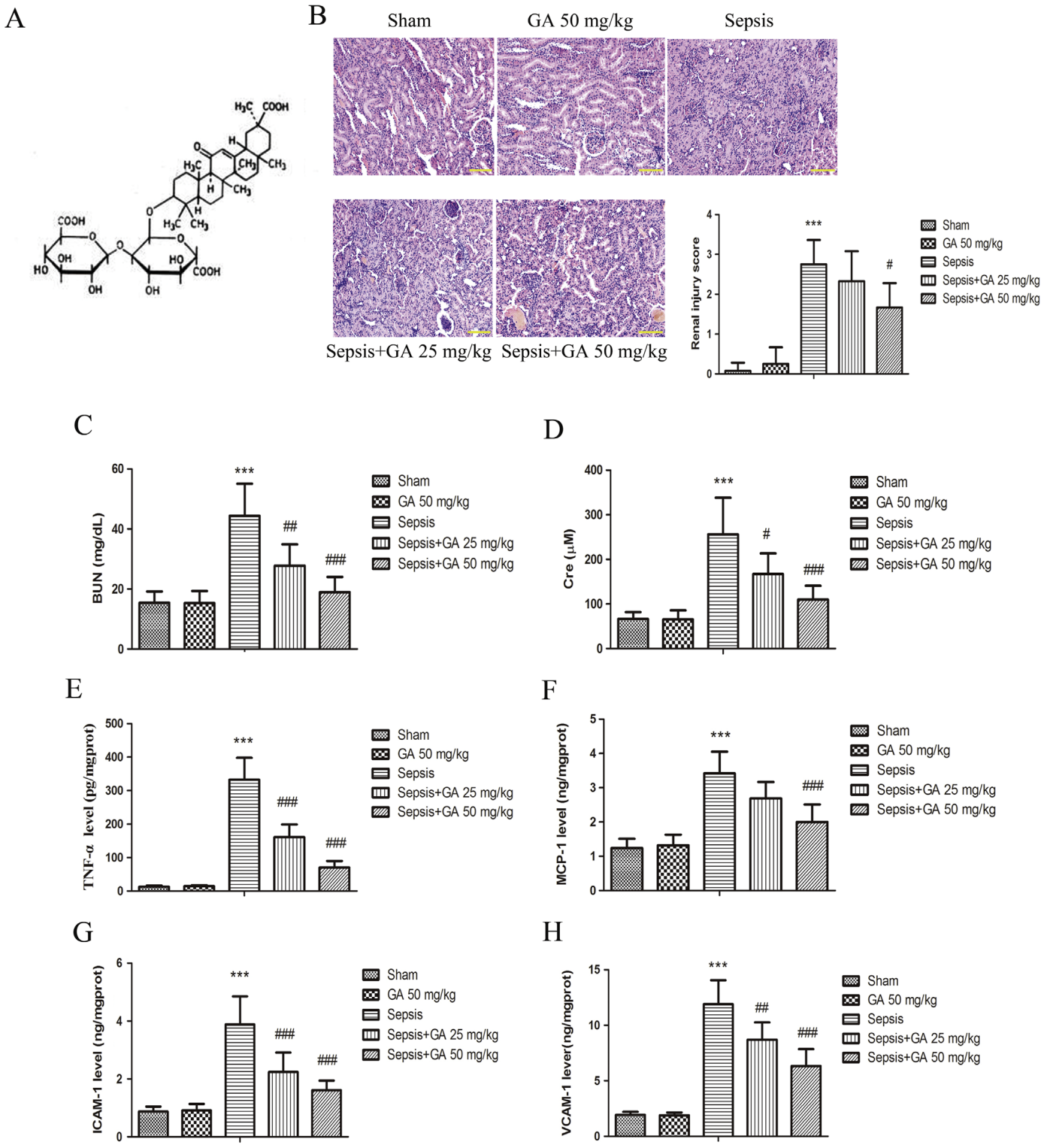

Fig. 1. GA alleviated sepsis-induced kidney injury *in vivo*. (A) Chemical structure of GA. The molecular formula of GA is $C_{42}H_{62}O_{16}$ and its molecular weight is 822.93. (B) The sepsis-induced histopathologic changes in kidney tissues were detected by HE staining assay (magnification 200×) and the renal injury score was shown. The concentrations of BUN (C), Cre (D) in peripheral blood from different groups. The TNF-α (A), MCP-1 (B), ICAM-1 (C), VCAM-1 (D) levels in kidney tissues were determined by ELISA. The results shown are representative of at least three independent experiments. Each value represents the mean ± S. D. (n=6).$^{***}P<0.001$, versus the sham operation group. $^{\#}P<0.05$; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$, versus the sepsis group.

showed in Fig. 1(A). The DMEM was used for diluting the drug for the different experiments. LPS (*Escherichia coli* serotype 055. B5) was purchased from Sigma (St. Louis, USA). The antibodies used for western blotting and immunofluorescence were purchased from WanLei Bioscience Co., Ltd (Shenyang, China).

2.3. Animals and experimental protocol

Male Sprague–Dawley rats (with an initial body weight of 200 ± 20 g) were purchased from Beijing Vital River Laboratory Animal Co., Ltd. (Beijing, China). The rats were fed freely at 23 °C