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Anti-inflammative effect of glycyrrhizin on rat thermal injury via inhibition of high-mobility group box 1 protein

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ABSTRACT

Aim: Glycyrrhizin (Gly) has been reported as an inhibitor of extracellular HMGB1 (high-mobility group box 1 protein) cytokine's activity, and protects spinal cord, liver, heart and brain against ischemia-reperfusion-induced injury in rats. The purpose of this study was to investigate the protective effect of Gly in rat skin thermal injury model and to elucidate the underlying mechanisms.

Methods: Twenty-four male Sprague-Dawley rats (200–250 g) were randomly divided into control group, vehicle-treated and Gly-treated burn groups, each group contained eight animals. In the latter two groups, rats were subjected to 30% TBSA (Total Body Surface Area) full-thickness scald injury. In Gly-treated burn group, glycyrrhizin (60 mg/kg) was administered intraperitoneally immediately after and at 24th hour burn; in vehicle-treated burn group, Ringer's solution (4 ml/kg, as a vehicle) was administered intraperitoneally immediately after and at 24th hour burn. The animals were sacrificed at 48 h after injury. Aortic blood samples were obtained to detect tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) with ELISA (Enzyme-Linked Immuno Sorbent Assay) kits. Lung, liver and kidney tissue samples were collected to determine the expression of HMGB1 mRNA and protein. HMGB1 mRNA level was semiquantitatively measured by Real-Time PCR using β-actin as an internal standard, and protein expression of HMGBI was determined by Western blot.

Results: Severe skin scald injury caused a significant increase in plasma TNF-α and IL-1β versus the control group ($P < 0.001$) in 48 h after burns. Intraperitoneal administration of Gly (60 mg/kg) significantly reduced the levels of serum TNF-α and IL-1β ($P < 0.01$). Gly treatment reduced these biochemical indices accompanied by lower level of HMGB1 protein ($P < 0.05$) and mRNA expression ($P < 0.01$).

Conclusion: These results demonstrate that Gly possesses an anti-inflammation effect to protect the remote organs from burn-induced injury.

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1. Introduction

Burn area greater than one-third of the total body surface area (TBSA) or full-thickness burn more than 10% of the total body surface area (TBSA) causes severe burn injury [1]. Severe skin thermal injury breaks the balance of the immune system which plays an important and complex role in the whole pathophysiologic process [2,3]. There are strong dependencies between mortality, morbidity and immune system imbalance [4].

As reported, the severe burn injury triggers the activation of an inflammatory cascade, resulting in excessive accumulation of potentially injurious pro-inflammatory mediators, such as IL -1, TNF-α, IL-6, prostaglandin E2 (PGE2), reactive nitrogen intermediates (RNI), transforming growth factor (TGF)-β) [4], however, as a matter of fact, some immune function is suppressed at the same time [5–7], just like immune responses of macrophagocyte, neutrophile granulocyte and lymphocyte become weak [8–10]. Immunologic derangement has a great effect on shock, sepsis and multiple organ dysfunction syndrome (MODS) [4]. Patients with extensive burns and sepsis can rapidly develop systemic inflammatory response syndrome (SIRS), many organs, just like the lung, liver, and kidney are damaged, which may cause multiple organ dysfunction syndrome (MODS) [11–13]. The various mediator in burn injury were produced and released by actived immunocytes and necrotic cells, the most noteworthy is overactive mononuclear macrophage, a key character in postburn immune [14]. It is of considerable clinical importance that the exact mechanism of mediator-induced, because it would allow for more deeply development of pharmacological modulation of burn sepsis, systemic inflammatory response syndrome (SIRS) by mediator inhibition.

Based on recent studies, it is now believed that high-mobility group box 1 (HMGB1) protein, an abundant non-histone nucleoprotein that acts as an architectural chromatin binding factor [15], plays an important role in postburn immune disorder. The HMG-1 was first purified from nuclei, and termed ''high mobility group'' (HMG) protein because of its rapid mobility on electrophoresis gels, and was renamed HMGB1 subsequently by a nomenclature committee [16]. The binding activity of HMGB1 to DNA is regulated by the two 80-amino acid DNA binding domains, the A-box and B-box, in addition, there is an acidic tail in the C-terminal of HMGB1. The B-box acts like pro-inflammatory factor which can induce macrophage secrete cytokines, while the A-box acts the opposite way [17]. HMGB1 contains three conserved redox-sensitive cysteines (C23, C45, and C106); modification of these cysteines determines the bioactivity of extracellular HMGB1 [18]. HMGB1 can be passively released by necrotic cells, cells infected by viruses [19–22] or mycobacteria [23,24], and actively released by innate immune cells in respond to endogenous host stimuli or exogenous bacterial products [25], and serves as a signaling molecule involve in acute and chronic inflammatory injury by binding to the receptor for advanced glycation end-products (RAGE) or Toll-like receptors (TLR2, 4 and 9), which results in the activation of pro-inflammatory pathways and enhanced inflammatory injury [15]. Therefore, the anti-inflammatory strategies based on targeting HMGB1 is of significant experimental and clinical interests.

Glycyrrhizin, a major and active constituent of roots of licorice, has long been known to exhibit glucocorticiod-like anti-inflammatory actions by inhibiting 11β-hydroxysteroid dehydrogenase [26], and is commonly used in Asia to treat patients with chronic hepatitis [27–29]. High dose of glycyrrhizin may leads to high blood pressure, salt and water retention, low potassium levels, and hormonal disbalances, for pregnant women, it can lead to a risk of preterm labor [30]. Recently, Gly was recognized as an HMGB1 inhibitor by Sitia et al. that acts by binding directly to both HMG boxes [31]. Gly has been used to interact with HMGB1 in ischemia-reperfusion animal model, the results indicated Gly alleviate HMGB1-associated damage [32]. However, no study has been designed to examine its use in preventing multiple organ derangement through HMGB1 pathway in severe skin burn injury. This study was designed to investigate the effects of Gly known as an inhibitor of HMGB1 on thermal injury rat model.

2. Materials and methods

2.1. Animals and groups

All experiments were performed in accordance with the ''Guide for the Care and Use of Laboratory Animals'' published by the US NIH (National Institutes of Health Publication No. 85-23, revised 1996) and were approved by the Committee for Animal Experiments at the Nanjing Medical University. Male Sprague-Dawley rats weighing 200–250 g were obtained from the Experimental Animal Center of Drum Tower Hospital (Nanjing, China) and were kept at a constant temperature (22 ± 1 °C) with 12 h:12 h light and dark cycles. They were fed with standard rat chow and were fasted for 12 h before the experiments, but were allowed free access to water. Rats were randomly divided into the following three groups: control group (n = 8), vehicle-treated burn group (n = 8) and Gly-treated burn group (n = 8).

2.2. Thermal injury and experimental design

Each rat was anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Dorsum of the animals was shaved, exposed to 99 °C water bath for 18 s to induce a third-degree burn involving 30% of the TBSA (TBSA = $K \times W^{0.5}$, K = 9.0, represent a constant; W represent the weight of the rat) [33]. The depth of burn was conformed by pathology. All animals were not given fluid resuscitation for the burn, but they were free to reach food and water. In Gly-treated burn group, glycyrrhizin (60 mg/kg) was administered intraperitoneally immediately after and at 24th hour burn; in vehicle-treated burn group, Ringer's solution (4 ml/kg, as a vehicle) was administered intraperitoneally immediately after and at 24th hour burn [34]. In both vehicle- and Gly-treated burn groups, rats were decapitated at 48 h following burn under sodium pentobarbital anesthesia. In order to rule out the effects of anesthesia, the same protocol was applied in the control group, except that the dorsum was dipped in a 25 °C