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Puerarin attenuates severe burn-induced acute myocardial injury in rats

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ABSTRACT

Background: Puerarin, the main isoflavone glycoside extracted from the root of *Pueraria lobata*, is widely prescribed for patients with cardiovascular disorders in China. This study investigates the effect of puerarin on severe burn-induced acute myocardial injury in rats and its underlying mechanisms.

Materials and methods: Healthy adult Wistar rats were divided into three groups: (1) sham group, sham burn treatment; (2) burn group, third-degree burns over 30% of the total body surface area (TBSA) with lactated Ringer's solution for resuscitation; and (3) burn plus puerarin group, third-degree burns over 30% of TBSA with lactated Ringer's solution containing puerarin for resuscitation. The burned animals were sacrificed at 1, 3, 6, 12, and 24 h after burn injury. Myocardial injury was evaluated by analyzing serum creatine kinase MB fraction (CK-MB) activity and cardiac troponin T (cTNT) level. Changes in cardiomyocyte ultrastructure were also determined using a transmission electron microscope. Tumor necrosis factor (TNF)-α concentration in serum was measured by radioimmunoassay. Cardiac myeloperoxidase (MPO) activity and malondialdehyde (MDA) concentration were measured to determine neutrophil infiltration and oxidative stress in the heart, respectively. The expression of p38 mitogen-activated protein (MAP) kinase in the heart was determined by Western blot analysis.

Results: After the 30% TBSA full-thickness burn injury, serum CK-MB activities and cTnT levels increased markedly, both of which were significantly decreased by the puerarin treatment. The level of serum TNF-α concentration in burn group at each time-point was obviously higher than those in sham group (1.09 ± 0.09 ng/ml), and it reached the peak value at 12 h post burn. Burn trauma also resulted in worsen ultrastructural condition, elevated MPO activity and MDA content in heart tissue, and a significant activation of cardiac p38 MAP kinase. Administration of puerarin improved the ultrastructural changes in cardiomyocytes, decreased TNF-α concentration in serum as well as suppressed cardiac MPO activity and reduced MDA content, and abolished the activation of p38 MAP kinase in heart tissue after severe burn.

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Conclusions: These results suggest that puerarin attenuates inflammatory responses, reduces neutrophil infiltration and oxidative stress in the heart, and protects against acute myocardial injury induced by severe burn.

1. Introduction

Acute myocardial injury is a common complication in patients with extensive burns in which the burned area exceeds 30% of the total body surface area (TBSA) and always persists for quite a long period [1]. During the early stage post burn, acute myocardial injury may exacerbate burn shock or cause refractory shock and can even lead to cardiac dysfunction [1,2]. Numerous experimental and clinical studies have stressed the importance of early cardiac myocyte damage during resuscitation [3,4]. However, acute myocardial injury is often overlooked by physicians because focus is usually concentrated on correcting the overwhelming state of hypovolemic shock and electrolyte abnormalities [5]. Although the pathophysiologic mechanisms underlying burn-induced acute myocardial injury remain incompletely elucidated, an increasing amount of evidence shows that systemic inflammatory response and oxidative stress resulting from thermal injury play important roles in the process [2,6–9].

Puerarin (4′-7-dihydroxy-8-β-D-glucosylisoflavone), the chemical structure of which being shown in Fig. 1, is a C-glycoside compound extracted from the root of *Pueraria lobata* (called Gegen in Chinese) [10]. It is a traditional Chinese medicinal herb widely used in China, Japan, Korea, and Southeast Asia for the treatment of angina pectoris, myocardial infarction, and other cardiac symptoms [10,11]. Previous studies have revealed that puerarin could increase coronary blood flow, decrease myocardial oxygen consumption, and protect against myocardial ischemic-reperfusion injury both in animals and in patients suffering from cardiovascular diseases [12–14]. However, here has been little information regarding the effect of puerarin on severe burn-induced acute myocardial injury.

Puerarin has been demonstrated to exert strong antioxidative activity in animals and humans with acute ischemic myocardial injury [10,14], liver injury[15], rheumatoid arthritis [16], diabetes [17], and so on. In addition, recent studies show that puerarin has a potential anti-inflammatory effect and may be a novel agent for managing rheumatoid arthritis or alcohol-induced liver injury [16,18,19]. Thus, we hypothesized that puerarin may protect against severe burn-induced acute myocardial injury by reducing systemic inflammatory response and/or oxidative stress. To test this hypothesis, we investigated the effect of puerarin on burn-induced myocardial injury as well as on systemic inflammatory cytokine release and cardiac oxidative stress in severely burned rats.

2. Materials and methods

2.1. Animals

Healthy adult Wistar rats (half were males and half were females) weighing 200–250 g were used throughout the study. All experimental manipulations were undertaken in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health and with the approval of the Animal Experimental Ethics Committee of Anhui Medical University, China. Animals were fed a standard animal diet with food and tap water *ad libitum* and acclimatized to their environment for at least one week prior to the experiment.

2.2. Burn procedure

The rats were anesthetized with pentobarbital (40 mg/kg) intraperitoneally, shaved on the dorsal and lateral surfaces, and secured on a constructed template device. The surface area of the skin exposed through the template device was immersed in 100 °C water for 12 s on the dorsal surface. All areas were quickly dried after each exposure to prevent additional injury. A 30% TBSA full-thickness dermal burn was then obtained using this technique [20].

2.3. Experimental design

A total of 80 rats were subjected to a 30% TBSA full-thickness burn and randomized into a burn group ($n = 40$) and a burn plus puerarin group ($n = 40$). The burn control rats were then resuscitated with an intraperitoneal injection of 4 ml/kg/TBSA of lactated Ringer's solution according to the Parkland formula. Puerarin (from Yangtze River Pharmaceutical Group, Taizhou, Jiangsu, China) was diluted 1:1200 (w/v) in the lactated Ringer's solution. The rats in the burn plus puerarin group were treated with 4 ml/kg/TBSA puerarin lactated Ringer's solution in the same manner. That is, the dose of puerarin given was 10 mg/kg. Subsequently, the burned animals were sacrificed at 1, 3, 6, 12, and 24 h after injury for blood and tissue sampling. A separate group of sham burned rats ($n = 8$) was subjected to an identical preparation except for immersion in room temperature water and fluid resuscitation.

2.4. Measurement of creatine kinase MB fraction (CK-MB) activity and cardiac troponin T (cTNT) level in serum

Myocardial injury was assessed by determining serum CK-MB activity and cTNT level. The activity of serum CK-MB was investigated using Vitros 250 automatic analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA), and the results were expressed in international units per liter. Serum cTNT level was measured using a radioimmunoassay (RIA) kit for rat cTNT (North Biotechnology Institute, Beijing, China) according to the manufacturer's instructions, and the results are expressed in nanogram per milliliter.

2.5. Cardiomyocyte ultrastructure examination

For examination by transmission electron microscopy, the myocardial tissue was fixed with 2.5% glutaraldehyde in