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Effects of glycine supplementation on myocardial damage and cardiac function after severe burn

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ABSTRACT

Background: Glycine has been shown to participate in protection from hypoxia/reoxygenation injury. However, the cardioprotective effect of glycine after burn remains unclear. This study aimed to explore the protective effect of glycine on myocardial damage in severely burned rats.

Methods: Seventy-two Wistar rats were randomly divided into three groups: normal controls (C), burned controls (B), and glycine-treated (G). Groups B and G were given a 30% total body surface area full-thickness burn. Group G was administered 1.5 g/(kg d) glycine and group B was given the same dose of alanine via intragastric administration for 3 d. Serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate transaminase (AST), and blood lactate, as well as myocardial ATP and glutathione (GSH) content, were measured. Cardiac contractile function and histopathological changes were analyzed at 12, 24, 48, and 72 hours.

Results: Serum CK, LDH, AST, and blood lactate increased, while myocardial ATP and GSH content decreased in both burned groups. Compared with group B, the levels of CK, LDH, and AST significantly decreased, whereas blood lactate as well as myocardial ATP and GSH content increased in group G. Moreover, cardiac contractile function inhibition and myocardial histopathological damage in group G significantly decreased compared with group B.

Conclusion: Myocardial histological structure and function were damaged significantly after burn. Glycine is beneficial to myocardial preservation by improving cardiomyocyte energy metabolism and increasing ATP and GSH abundance.

1. Introduction

Ischemic/hypoxic damage induced by burn stress is a major cause of heart injury after severe burn. Our previous study showed that regional myocardial blood flow and myocardium ATP synthesis decrease significantly in the early stages of severe burn [1,2]. To date, an effective therapeutic measure for myocardial protection in burn patients has not been found. Fluid resuscitation and cardiotonic drugs are currently the main therapeutic methods after burn [3–5]. Fluid infusion may increase blood volume and improve hemodynamic parameters. However, low cardiac output and myocardial hypoxia–ischemia are not completely resolved. Excessive fluid infusion may aggravate cardiac preload and increase myocardial damage. Therefore, fluid infusion cannot

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fundamentally correct cardiac muscle ischaemia and alleviate cardiac muscle damage [6–9]. Cardiotonic drugs can improve myocardial contractility and partly improve the derangement of blood stream dynamics. However, they cannot fundamentally repair the imbalance in oxygen supply and consumption in cardiac myocytes [10]. Therefore, discovery of therapeutic regimens is necessary for improving energy metabolism, reducing myocardial cell damage, and ameliorating myocardial function.

In recent years, many studies found that glycine protects mammalian cells against ischemic injury and accelerate cellular recovery by preventing cellular membrane leakage, inhibiting cell calcium overload, and improving mitochondrial function [11,12]. Several studies have proven that the glycine receptor is expressed in cardiomyocytes and participates in cytoprotection from hypoxia/reoxygenation injury [11,13,14]. Glycine protects cardiomyocytes against ischemia–reperfusion (IR) injury by inhibiting mitochondrial permeability transition [15]. Therefore, glycine is an important myocardial cytoprotective agent. However, the cytoprotective effect of glycine on cardiocytes after burn is rarely studied. The present study evaluated the therapeutic effect of glycine on rat cardiac damage induced by severe burn and explored the possible mechanisms.

2. Materials and methods

2.1. Drugs and reagents

Glycine and ATP were obtained from Sigma Chemical Co. (St. Louis, MO). Blood lactic acid and glutathione (GSH) detection kits were obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Alanine injections were purchased from Fujian Haiwang Pharmaceutical Ltd. (Fuzhou, China). All other chemicals and reagents were of analytical grade.

2.2. Experimental animals

Seventy-two male adult Wistar rats weighing 200–245 g were offered by the Laboratory Animal Center, Third Military Medical University. The rats were placed in individual wire-bottomed cages under controlled temperature and humidity with a 12 h light–dark cycle. The rats were acclimatized to the environment with a diet of standard rat pellets for 7 d prior to the experiment. The rats were then randomly divided into three groups: normal control (C), burned control (B), and glycine-treated (G) groups. Eight rats from group C were shaved and anesthesized but not burned. Sixty-four burned rats from groups B and G were inflicted with 30% total body surface area of full-thickness burn under general anesthesia (pentobarbital, 40 mg/kg of body weight) and analgesia (buprenorphine, 1 mg/kg of body weight) following a modified procedure as previously described [2]. The rats were anesthetized, shaved, napalm-burned for 18 s, and intraperitoneally injected with lactated ringer's solution (1.5 ml/kg per 1% of burned body surface area) for resuscitation. The eight rats from each group at each phase were observed at four time points: 12, 24, 48, and 72 post-burn hours (PBH).

The experiments in the current study conforms to the regulations stipulated by the Third Military Medical University Animal Care Committee, according to the protocol outlined in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication no. 85-23, revised 1996).

2.3. Medicinal treatments

Groups G and B were supplemented with 1.5 g/(kg d) glycine or alanine for 3 d via intraperitoneal injection, respectively. All medicines were administered twice at an interval of 24 h. The rats were housed in solitary cages and allowed to have free access to food and water. At each observed phase, cardiac contractile function index was measured; the blood plasma and myocardial tissue were harvested and conserved at −80 °C or in liquid nitrogen, respectively.

2.4. Histological examination

The harvested cardiac apex was fixed in neutral formalin solution (pH 7.4) and wax embedded. Serial sections were then performed for microscopic study. Hematoxylin–eosin (HE) stains were used to reveal histological changes.

2.5. Myocardial zymogram

The blood samples were centrifuged at 4000 rpm for 10 min at 4 °C, and the serum fraction was transferred into another clean tube. Myocardial injury was assessed by determining the levels of serum creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) using an auto-analyzer AU-800 (Olympus, Japan). The results were expressed in international units per liter.

2.6. Lactic acid content in blood and GSH content in tissues

The lactate content in blood was measured using a blood lactic acid detection kit and a 721 spectrophotometer (Beckman, US). Blood (0.1 ml) was processed with 0.6 ml of a protein precipitant. The mixture was centrifuged at 4000 rpm for 8 min at 4 °C. The procedure was performed according to the kit guidelines. Color reaction was monitored by measuring the absorbance at 530 nm. The results were expressed as millimole lactic acid per liter of whole blood.

The content of GSH in the tissue was determined using a GSH detection kit and a 721 spectrophotometer. Approximately 100 mg of myocardium tissue was homogenated with 5% sulfosalicylic acid and 1 ml of phosphate-buffered saline solution. The homogenate was kept on ice for 30 min and then centrifuged at 18,000 rpm for 20 min at 4 °C. Total GSH concentrations were determined by adding 2-nitrobenzoic acid into appropriate aliquots of the supernate. The procedure was performed according to the kit guidelines. Color reaction was monitored by measuring the absorbance at 412 nm. Protein concentration of the supernate was determined using a BCA reagent (Pierce, US). The results were expressed as micromole GSH per gram protein.