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# Pretreatment with hydrogen-rich saline reduces the damage caused by glycerol-induced rhabdomyolysis and acute kidney injury in rats

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ABSTRACT

*Background:* Rhabdomyolysis is a leading cause of acute kidney injury. The pathophysiological process involves oxidative stress and inflammation. Hydrogen-rich saline (HRS) is an antioxidant and anti-inflammatory. This study explored the protective effect of pretreatment with HRS on the development of glycerol-induced rhabdomyolysis acute kidney injury.

*Materials and methods:* Forty-eight rats were randomly divided into four equal groups. Group 1 served as the control, group 2 was given 50% glycerol (10 mL/kg, intramuscular), group 3 was given glycerol after 7 d pretreatment with high dose HRS (10 mL/kg/d, intraperitoneal), and group 4 was given glycerol after 7 d pretreatment with low dose HRS (5 mL/kg/d, intraperitoneal). Renal health was monitored by serum creatinine (Cr), urea, and histologic analysis; rhabdomyolysis was monitored by creatine kinase (CK) levels; and oxidative stress was monitored by kidney tissue reactive oxygen species (ROS), malondialdehyde, 8-hydroxydeoxyguanosine (8-OH-dG), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX) levels. Inflammation was monitored by interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) evaluation.

*Results:* Glycerol administration resulted in an increase in the mean histologic damage score, serum Cr, urea and CK, kidney tissue ROS, malondialdehyde, 8-OH-dG, GSH-PX, IL-6, and TNF-α, and a decrease in kidney tissue superoxide dismutase activity. All these factors were significantly improved by both doses of HRS, but the mean histologic damage score, urea, Cr, CK, ROS, 8-OH-dG, GSH-PX, IL-6, and TNF-α for the high dose HRS treatment group were even lower.

*Conclusions:* Pretreatment by HRS ameliorated renal dysfunction in glycerol-induced rhabdomyolysis by inhibiting oxidative stress and the inflammatory response.



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## 1. Introduction

Rhabdomyolysis refers to the breakdown of striated muscle, which results in the release of potentially toxic compounds into the circulation that may affect kidney function. Rhabdomyolysis is one of the most common reasons for acute kidney injury (AKI) and accounts for 15% of cases with a mortality of 5% [1]. Rhabdomyolysis can have many causes such as crush injury, medications, infections, myopathies and muscular dystrophies, and various other diseases. A common cause is excessive training, this is called exertional rhabdomyolysis and is often seen in military training [2,3]. Rhabdomyolysis caused AKI in 51% of cases in a hospital study, with 32% fatalities [4]. The most widely used model of myoglobinuric acute renal failure is by intramuscular injection of hypertonic glycerol [5]. This, when utilized in rats produces myoglobinuria and the resulting responses typical of the human rhabdomyolysis syndrome.

The oxidative stress damage caused from the free-iron-catalyzed Fenton reaction, myoglobin redox cycling, and generation of oxidized lipids can lead to rhabdomyolysis renal failure [6,7]. The inflammatory reaction also participates in rhabdomyolysis AKI [8,9]. The standard treatment for rhabdomyolysis AKI is an aggressive rehydration, and this might be sufficiently effective for patients with a mild form of the disease. However, the evidence suggests that pharmacologic agents that inhibit myoglobin redox cycling might represent the best therapeutic intervention for patients with a more severe form of this disease [6]. Studies have shown that vitamin C [10–12], alpha melanocyte–stimulating hormone [13], montelukast [14], resveratrol [15], and L-carnitine [16,17] can protect against rhabdomyolysis AKI by rectifying detrimental changes in the antioxidant profile and systemic cytokines. At present, the studies are largely on vitamin C. Oxidative metmyoglobin, the oxidized form of myoglobin, is a toxic molecule that triggers oxidative stress reactions, such as lipid peroxidation, that lead to muscle ischemia–reperfusion injury [7]. Vitamin C has been shown to be able to effectively inhibit the formation of metmyoglobin [11] and appears to be a promising candidate for the prevention of rhabdomyolysis AKI [18]. However, vitamin C can make urine weakly acidic and reducing urine pH even slightly is adverse for patients. In addition, vitamin C reacts with free radicals, which can produce toxic metabolites that subsequently need to be removed.

In 2007, Ohsawa *et al.* [19] for the first time showed that a low dose of hydrogen ($H_2$) can significantly improve rats after stroke, and proved that the inhalation of $H_2$ gas markedly suppressed brain injury by buffering the effects of oxidative stress. Later, they also proved that inhalation of 2% $H_2$ can treat reperfusion injury in the liver and myocardium [20,21]. Because of the great limitations of breathing the drug, some scholars applied special equipment to prepare hydrogen-rich saline (HRS) [22,23]. It has been shown that HRS can protect rats from ischemic brain injury and diabetic retinopathy [24,25]. It has since been demonstrated using animal models that HRS may be beneficial to a wide range of diseases and ailments [26], including renal ischemia–reperfusion injury [27,28], ischemia-induced cardiorenal injury [29], cisplatin-induced nephrotoxicity [30–32], and chronic allograph nephropathy [33]. Dissolved $H_2$ has also been studied in a clinical trial for its effectiveness in preventing chronic inflammation during hemodialysis [34]. So it is possible that HRS could also be a valuable tool against rhabdomyolysis AKI. HRS has been found to be a safe and effective antioxidant [19] and anti-inflammatory [20]. Compared with the traditional antioxidants, $H_2$ has several advantages. $H_2$ can easily penetrate biomembranes and diffuse into the cytosol, mitochondria, and the nucleus because of its low molecular weight; it is mild enough not to disturb metabolic oxidation–reduction reactions or disrupt reactive oxygen species (ROS) mediated cell signaling. Many animal experiments have confirmed the antioxidant effect of the HRS [26], for example, Ono *et al.*[35] administrated 500 mL HRS to four patients and improved acute erythemtous skin diseases.

We hypothesized that pretreatment with HRS could protect against rhabdomyolysis AKI by antioxidant and anti-inflammatory methods. We applied the glycerol-induced rhabdomyolysis AKI rat model to validate HRS by the way of its antioxidant and anti-inflammatory protection against rhabdomyolysis AKI.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats, specific pathogen free, weighing 180–200 g, were bought from Shandong University of Traditional Chinese Medicine, and bred in Mount Taishan Medical University Animal Center. The rats were fed with conventional rat feed, free feeding and drinking. They were housed in an air-conditioned room with 12 h light–dark cycles, where the temperature (21 ± 2°C) and relative humidity (60%–65%) were kept constant. The study protocol was approved by the Ethics Committee of No.88 Hospital of PLA.

### 2.2. HRS production

HRS was prepared as previously described [25]. $H_2$ gas was dissolved in physiological saline for 2 h under high pressure (0.4 MPa) to a supersaturated level using HRS producing apparatus made by the Institute of Atherosclerosis, Taishan Medical School, China. The saturated HRS was stored under atmospheric pressure at 4°C in an aluminum bag with no dead volume. HRS was sterilized by gamma radiation. HRS was freshly prepared every week, which ensured that a concentration of 0.6 nmol/L was maintained.

### 2.3. Study design

Rats were randomly divided into four groups, each comprising of 12 animals. The animals were allowed free access to food, but deprived of drinking water for 24 h before glycerol injection.

Group 1 serves as the control group. The animals were treated with saline (10 mL/kg/d, intraperitoneal [i.p.]) for 7 d, deprived of drinking water for 24 h on the sixth day, then were