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Plasma glutathione reductase activity and prognosis of septic shock

Jae Seong Kim, MD,[a] *Woon Yong Kwon, MD, PhD,*[a,*] *Gil Joon Suh, MD, PhD,*[a] *Kyung Su Kim, MD, PhD,*[a] *Yoon Sun Jung, MD,*[b] *Sung Hee Kim, MSc,*[a] *and So Eun Lee, MD*[c]

[a] *Department of Emergency Medicine, Seoul National University College of Medicine, Seoul, Korea*
[b] *Department of Emergency Medicine, National Medical Center, Seoul, Korea*
[c] *Department of Emergency Medicine, Incheon Sarang Hospital, Incheon, Korea*

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ABSTRACT

Background: Our aim was to investigate whether plasma glutathione reductase (GR) activity is well correlated with the erythrocyte-reduced glutathione (GSH)/glutathione disulfide (GSSG) ratio and is associated with the mortality of septic shock.

Materials and methods: This study was conducted on male Sprague–Dawley rats and patients admitted to the intensive care unit with septic shock. To induce endotoxemia in rats, vehicle or lipopolysaccharide (LPS) at dosages of 5 or 10 mg/kg were injected into a tail vein. Animals were then euthanized 6 h post-LPS. Based on the 28-d mortality, the enrolled patients were divided into the survivors and nonsurvivors. We obtained blood samples from patients at admission (0 h) and 24 h after admission to the intensive care unit.

Results: In endotoxemic rats, the erythrocyte GSH/GSSG ratio, erythrocyte GR activity, and plasma GR activity in the 10 mg/kg of LPS group were lower than those in the sham and 5 mg/kg of LPS groups. In patients with septic shock, decrease in plasma GR activity at 24 h was independently associated with an increase in 28-d mortality (odds ratio, 0.828; 95% confidence interval, 0.690–0.992, $P = 0.041$). Plasma GR activity was correlated with erythrocyte GR activity (Spearman $\rho = 0.549$, $P < 0.001$) and the erythrocyte GSH/GSSG ratio (*rho* $= 0.367$, $P = 0.009$) at 24 h.

Conclusions: Plasma GR activity was well correlated with erythrocyte GR activity and the erythrocyte GSH/GSSG ratio, and a decrease in plasma GR activity was associated with an increase in the mortality of septic shock patients.

1. Introduction

1.1. Background

Sepsis is defined as a complex clinical syndrome that is caused by a harmful or damaging host response to infection and may progress to the development of multiple-organ dysfunctions and death [1]. Sepsis activates various immune cells and leads to the excessive production of reactive oxygen species (ROS) [2]. The ROS and ROS–derived oxidative stresses appear to be implicated in cellular signal transduction and gene activation, leading to the excessive or inappropriate release of inflammatory mediators and the development of multiorgan failure during sepsis [3–5].

* *Corresponding author*. Department of Emergency Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 110 744, Korea. Tel.: +82 2 2072 0326; fax: +82 2 3672 8871.
E-mail address: kwy711@hanmail.net (W.Y. Kwon).
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Oxidative stresses occur when the balance between the production of ROS and their removal by endogenous antioxidant defenses are not maintained [6]. Circulating erythrocytes scavenge extracellular ROS and provide an antioxidant defense for the surrounding tissues [7,8]. Glutathione (GSH) is a major intracellular antioxidant that eliminates ROS [3], and previous studies have reported that oxidative stresses are potentiated by the depletion of reduced GSH during sepsis [9–11]. Intracellular GSH synthesis is mainly regulated by glutamate-cysteine ligase (GCL) activity [12,13]. In erythrocytes, the GSH redox cycle is regulated by glutathione peroxidase (GPx) and glutathione reductase (GR). Briefly, GPx eliminates hydrogen peroxide (H_2O_2) through the oxidation of GSH to glutathione disulfide (GSSG) [3,14,15]. GR then reconverts GSSG to GSH [3,14,15]. Therefore, erythrocyte GSH redox (GSH/GSSG) ratio is shown to be a sensitive parameter of antioxidant capacities [3,11,16]. To measure the erythrocyte GSH/GSSG ratio, erythrocytes should be separated from blood samples in real time, and many researchers tried to estimate the erythrocyte GSH/GSSG ratio by whole blood or plasma. Previous clinical studies showed that the blood GSH/GSSG ratio was decreased in patients with septic shock [11]. However, GSH in extracellular areas undergoes spontaneous autoxidation and GSSG formation, and accumulated GSSG in erythrocytes is exported into the plasma [17]. And thus, the blood GSH/GSSG ratio is not fully consistent with the erythrocyte GSH/GSSG ratio, and the erythrocyte GSH/GSSG ratio may not be properly estimated in plasma [13,16].

Among two enzymes which regulate the GSH redox cycle, decreases in plasma GPx activity and the selenium level are known to be associated with poor prognosis of septic shock. Selenium is an essential trace element to synthesize GPx, and previous studies showed that selenium suppressed oxidative stresses by increasing GPx activity [18–20]. However, GPx–induced H_2O_2 elimination needs the oxidation of GSH to GSSG. To restore the GSH level and maintain the GSH/GSSG ratio, GSSG should be reduced to GSH. GR catalyzes the reduction of GSSG to GSH mainly in intracellular area and is also secreted into extracellular area and protects cells from external oxidative stresses [16,21]. Furthermore, plasma GR activity can be easily measured by spectrophotometry in a clinical setting. However, there have been limited data which investigate whether plasma GR activity is well correlated with the erythrocyte GSH/GSSG ratio and is associated with prognosis of septic shock.

1.2. Hypotheses and purpose

Therefore, we hypothesized that erythrocyte GR activity would be well correlated with the erythrocyte GSH/GSSG ratio, plasma GR activity would be well correlated with the erythrocyte GR activity, and thus plasma GR activity would be well correlated with the erythrocyte GSH/GSSG ratio. We also hypothesized that a decrease in plasma GR activity would be associated with an increase in the mortality of patients with septic shock. To verify our hypothesis, we performed this study.

2. Materials and methods

2.1. Ethics statement

All the conducted animal experiments were approved by the Institutional Animal Care and Use Committee of Seoul National University Hospital (IACUC Number: 13-0393-S1A0) in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

We obtained clinical data and blood samples from the consecutive patients with septic shock who enrolled in a prospective cohort study, the repository for sepsis and postresuscitation samples (NCT01670383), from September 2012–April 2013. This study was performed in compliance with the Declaration of Helsinki (Seoul, Korea, 2008) and was approved by the Institutional Review Board of Seoul National University College of Medicine/Seoul National University Hospital (institutional review board number: 1012-140-347 and 1312-028-539). All the patients or their representatives provided written informed consent.

2.2. Animals and drugs

The experiments were performed on male Sprague–Dawley rats (body weight, 300–350 g) purchased from Orient Bio Inc (Seongnam, Korea). Estrogen is known to influence pathophysiology and prognosis of sepsis [22,23]. Therefore, we did not use female gender rats in animal experimental study to avoid confounding effects by different phases of estrous cycle. However, in the clinical study, we collected data from both the male and female septic shock patients. The animals were provided with a laboratory chow (Lab Diet, Seongnam, Korea) and water *ad libitum* and were housed in a specific pathogen-free room at constant temperature (20°C–22°C) with 10 and 14 h of light and dark exposure, respectively. The animals underwent an acclimatization period of 14 d before being used in the experiments.

Lipopolysaccharide (LPS, from *Escherichia coli*, O26:B6) was purchased from Sigma–Aldrich Chemical (St Louis, MO).

2.3. Animal experimental procedures

To induce endotoxemia in rats, vehicle or LPS at dosages of 5 or 10 mg/kg were injected into a tail vein. The animals were then returned to their cages and were allowed food and water *ad libitum*. The subjects were divided into three groups as follows: (1) the rats in the sham group were administered a vehicle intravenously; (2) the rats in the low dose of LPS (LD-LPS) group were administered 5 mg/kg of LPS intravenously; and (3) the rats in the high dose of LPS (HD-LPS) group were administered 10 mg/kg of LPS intravenously.

For the survival analysis, the animals were allocated to the sham group ($n = 6$), the LD-LPS group, or the HD-LPS group ($n = 11$ per group) and were closely observed for the occurrence of mortality during a period of 72 h. To evaluate the effects of low and high doses of LPS, a separate set of animals was allocated to the study groups based on the survival study findings: the sham group ($n = 6$), the LD-LPS group ($n = 8$), and