INCREASED SERUM CATALASE ACTIVITY IN RATS SUBJECTED TO THERMAL SKIN INJURY

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Abstract—We found that rats subjected to thermal skin injury (burn) had increased serum hydrogen peroxide (H$_2$O$_2$) scavenging activity, serum catalase activity, erythrocyte (RBC) fragility, and edematous lung injury (lung leak) when compared to sham-treated rats. Serum H$_2$O$_2$ scavenging activity was inhibited by addition of sodium azide, a catalase inhibitor. Treatment of rats with the oxygen radical scavenger, dimethylthiourea (DMTU), decreased RBC fragility and lung leak but did not alter increased H$_2$O$_2$ scavenging or catalase activity of serum from rats subjected to skin burn. We conclude that increased serum catalase activity is a consequence of thermal skin injury and that increased serum catalase activity may be a mechanism that modulates H$_2$O$_2$-dependent processes following skin burn.

INTRODUCTION

Although respiratory failure is a frequent and devastating complication in burn patients (1, 2), the mechanisms underlying its development remain unknown. Several studies have implicated increased production of reactive oxygen species (ROS), such as superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (·OH), in the pathogenesis of lung injury following thermal

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skin injury (burn) (3–5), but potentially protective antioxidant enzyme defenses have not been evaluated following skin burn. Recently, we found that H₂O₂ scavenging activity and serum catalase activity were increased in patients with the adult respiratory distress syndrome (ARDS) (6). Based on these two observations, we hypothesized that H₂O₂ scavenging activity and catalase activity would increase in serum of rats subjected to skin burn. The results of the present investigation support this premise.

**MATERIALS AND METHODS**

*Source of Reagents.* Hanks' balanced salt solution (HBSS) was purchased from Gibco Laboratories (Grand Island, New York), hydrogen peroxide (H₂O₂) from J.T. Baker Chemical Co. (Phillipsburg, New Jersey), and DMTU from Aldrich Chemical Co. (Milwaukee, Wisconsin). All other reagents were obtained from Sigma Chemical Co. (St. Louis, Missouri).

*Skin Burn Protocol.* Adult female Sprague-Dawley rats (Sasco, Omaha, Nebraska) were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg). Subsequently, skin over the dorsal lumbosacral area was shaved and exposed to 70°C (burn) or 30°C (sham) water for 30 sec. The former caused a 30% second-degree burn. Rats remained anesthetized and had their temperature monitored on a warming blanket until blood or serum collection, which occurred 2 h after thermal injury. Blood and serum were obtained by intracardiac puncture. Some rats received dimethylthiourea (DMTU, 250 mg/kg, intraperitoneal) 1 h prior to thermal injury (7). Control rats received no treatment and were anesthetized immediately prior to blood and serum acquisition. Values for control and sham-treated rats were similar (P > 0.05) and pooled for comparison to burn treatment.

*Measurement of Serum H₂O₂ Scavenging Activity.* To assess serum H₂O₂ scavenging activity, samples of rat serum (0.1 ml) were mixed with H₂O₂ (0.1 ml of 10 mM) in a total reaction mixture of 1.0 ml of HBSS at 37°C for 30 min. In certain experiments, sodium azide (0.1 ml of 10 mM) was added before addition of H₂O₂. Subsequently, 0.1 ml of 50% trichloroacetic acid (TCA) was added to stop H₂O₂ consumption. After samples were centrifuged for 5 min, each supernatant (0.2 ml) was obtained and assayed in triplicate for residual H₂O₂ (8) and compared to a serum-free control sample.

*Measurement of Serum Catalase Activity.* Serum catalase activity was measured polarographically as the rate of production of oxygen from H₂O₂ (9). One unit of catalase activity was defined as the amount of catalase that consumed 1 μmol H₂O₂/min at 25°C, pH 7.0.

*Measurement of Erythrocyte (RBC) Fragility.* Packed RBC (20 μl) were separated from fresh heparinized blood, washed twice with normal saline, and added to 2.0 ml of saline of varying tonicity (0-0.9%). After gentle mixing, incubation at 25°C for 30 min, and centrifugation, supernatants were obtained and absorbance was measured at 414 nm. An index of RBC fragility was defined as the tonicity of saline causing 50% hemolysis.

*Measurement of Lung Leak.* An index of lung leak was measured as the accumulation in blood-free lungs of intravenously injected ¹²⁵I-labeled albumin (10).

*Statistical Analysis.* Treatment groups were compared using an analysis of variance with a Student–Newman–Keuls test of multiple comparisons. Significance was accepted at a P value of < 0.05.
RESULTS

Effect of Thermal Skin Injury (Burn) on Serum H$_2$O$_2$ Scavenging and Catalase Activity. Rats subjected to skin burn had increased ($P < 0.05$) serum H$_2$O$_2$ scavenging activity in vitro (Figure 1A) and increased ($P < 0.05$) serum catalase activity (Figure 1B) compared to sham-treated rats. Treatment with sodium azide, a catalase inhibitor, decreased in vitro H$_2$O$_2$ scavenging ability.

Fig. 1. (A) Serum H$_2$O$_2$ scavenging activity was increased ($P < 0.05$) in burn-treated rats compared to sham-treated rats. Rats pretreated with dimethylthiourea (DMTU) followed by burn treatment had increased ($P < 0.05$) serum H$_2$O$_2$ scavenging activity compared to DMTU-pretreated and sham-treated rats. (B) Serum catalase activity was increased ($P < 0.05$) in burn-treated rats compared to sham-treated rats. Rats pretreated with dimethylthiourea (DMTU) followed by burn treatment had increased ($P < 0.05$) serum catalase activity compared to DMTU-pretreated and sham-treated rats. (C) Red blood cell (RBC) fragility was increased ($P < 0.05$) in burn-treated rats compared to sham-treated rats. In contrast, rats pretreated with DMTU followed by burn treatment had similar ($P > 0.05$) RBC fragility compared to DMTU-pretreated and sham-treated rats. (D) Lung leak was increased ($P < 0.05$) in burn-treated rats compared to sham treated rats. In contrast, rats pretreated with DMTU followed by burn treatment had similar ($P > 0.05$) lung leak compared to DMTU-pretreated and sham-treated rats. All values are the mean ± SEM of eight determinations.
of rat serum by more than 90% (data not shown). Pretreatment of rats with DMTU had no effect on serum H₂O₂ scavenging or catalase activity of sham or burn-treated rats.

Effect of Thermal Skin Injury on RBC Fragility. The fragility of RBC isolated from burn-treated rats was increased \((P < 0.05)\) compared to RBC from sham-treated rats (Figure 1C). RBC from rats treated with DMTU before skin burn did not develop increases in fragility, which occurred in RBC from burn-treated rats that did not receive DMTU (Figure 1C).

Effect of Thermal Skin Injury on Lung Leak. Rats subjected to thermal skin injury had increased lung leak compared to sham-treated rats (Figure 1D). Rats treated with DMTU before skin burn did not develop increases in lung leak seen in burn-treated rats that did not receive DMTU (Figure 1D).

DISCUSSION

In the present investigation, we found that serum from rats subjected to thermal skin injury (skin burn) had increased H₂O₂ scavenging and catalase activity compared to sham-treated rats. Serum H₂O₂ scavenging activity appeared to be due to serum catalase activity, since it was inhibited by treatment with sodium azide, a catalase inhibitor. Increases in serum H₂O₂ scavenging and catalase activity were associated with increased RBC fragility and lung leak in rats subjected to skin burn.

In an attempt to determine the source of the increased serum catalase activity, we treated rats with an oxygen radical scavenger before initiating skin burn. We found that pretreatment with DMTU decreased RBC fragility and lung leak but did not alter increases in serum H₂O₂ scavenging or catalase activity in rats subjected to skin burn. These findings suggest that oxygen radicals contribute to RBC fragility and lung leak, but not to increases in serum H₂O₂ scavenging and catalase activity. The findings do not support the possibility that RBC or lung tissue destruction are the source(s) of increased serum catalase activity. The source of serum catalase remains unclear. It is possible that serum catalase is derived directly from the burned skin tissue.

It is known that thermal skin injury causes acute edematous lung injury, erythrocyte hemolysis, complement activation, lung neutrophil accumulation, as well as increases in serum xanthine oxidase (XO) activity and histamine in rats (4, 5, 11–13). Since lung injury following thermal injury is prevented by treatment with antioxidants, neutrophil depletion, or XO inhibition, it has been concluded that thermal injury most likely leads to systemic complement activation, neutrophil activation and accumulation in the lung, and the development of acute lung injury that is related to the increased production of toxic oxygen
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products by activated blood neutrophils and/or XO. It has also been shown that intravenous treatment with exogenous catalase decreases lung injury in rats subjected to thermal injury. This suggests that exogenous increases in serum catalase may alter the development of lung injury in rats subjected to thermal skin injury. However, the increases in serum catalase that occur naturally are much less than those achieved following treatment with exogenous catalase and obviously are not sufficient to prevent lung injury. Whether the increased endogenous serum catalase activity following skin burn limits lung injury, or any other \( \text{H}_2\text{O}_2 \)-dependent process, in any way or just occurs as a reflection of thermal injury remains speculative. The mechanism through which DMTU protected from lung leak is unclear but may involve scavenging of oxygen radicals (7).

Serum catalase is present in healthy human subjects in the range of 4–16 units/ml (9). In addition, increased levels of serum catalase activity have been observed in patients with ARDS (6) and AIDS (14). In each case, the source and significance of increased serum catalase activity is unclear. However, increased serum catalase activity appears to effectively predict the development of ARDS in patients with sepsis (6), and serum catalase activity increases progressively with advancing human immunodeficiency virus (HIV) infection (i.e., asymptomatic HIV infection < symptomatic HIV infection < AIDS) (14). The exact nature and consequences of increased serum catalase activity remain to be determined following skin burn and with respect to the development of ARDS, AIDS, and other processes that may involve \( \text{H}_2\text{O}_2 \). It is conceivable that serum catalase can penetrate microenvironments, such as the neutrophil–endothelial cell interface, that are inaccessible to catalase in the RBC (15). Furthermore, the possibility exists that even small increases in serum catalase can alter \( \text{H}_2\text{O}_2 \)-dependent processes under certain circumstances.

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