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THE INTERRELATIONSHIP OF SUPEROXIDE DISMUTASE AND PEROXIDATIC ENZYMES IN THE RED CELL

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Summary

Activities of superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1) and catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6) were determined during the course of incubation of red cell suspensions with 1,4-naphthoquinone-2-sulfonic acid. In the absence of glucose, incubation with napthoquinone sulfonate resulted in an inhibition of catalase and superoxide dismutase. The catalase inhibitor, 3-amino-1,2,4-triazole enhanced inactivation of catalase in the presence of naphthoquinone sulfonate and this in turn led to augmented inhibition of superoxide dismutase. The presence of glucose in the incubation medium prevented napthoquinone sulfonate-induced enzyme inhibition in the absence of aminotriazole, but had little effect in the presence of aminotriazole. The relevance of these findings to the cellular interrelationship of peroxidatic enzymes and superoxide dismutase is discussed.

Introduction

Oxygen-utilizing organisms possess a number of enzyme capable of destroying toxic reactive intermediates of oxygen metabolism. These enzymes, which include the superoxide dismutases (superoxide:superoxide oxidoreductases, EC 1.15.1.1), catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6) and the peroxidases are postulated to protect aerobic cells by scavenging such reactive oxygen intermediates as superoxide anion and hydrogen peroxide.

Primary defense mechanisms of the red cell against hydrogen peroxide are mediated through glutathione peroxidase [1—3] and catalase [4], while superoxide dismutase affords protection against superoxide anion [5,6]. It has been demonstrated that hydrogen peroxide inactivates purified superoxide dismutase

[7,8]; this observation has led us to postulate that peroxide-destroying enzymes may function to protect superoxide dismutase from inactivation by hydrogen peroxide.

In the studies presented, red cells were incubated with 1,4-napthoquinone-2-sulfonic acid, a water soluble analogue of an in vivo hemolytic drug, menadione. The activities of superoxide dismutase and catalase were determined in the presence of naphthoquinone sulfonate, with and without glucose and the catalase inhibitor, 3-amino-1,2,4-triazole, in order to examine the relationship between hydrogen peroxide, peroxide-destroying enzymes and superoxide dismutase.

Materials and Methods

Human adult blood obtained by venipuncture was drawn into 3.8% sodium citrate solution. Plasma and buffy coat were removed, followed by three washings of red cells in 0.9% NaCl. All red cell suspensions were 0.7% in 0.01 M sodium phosphate buffer, 0.144 M NaCl, 10^{-4} M EDTA, pH 7.4, and were incubated at 37°C either with or without 0.01 M glucose for 1.5 h prior to addition of any other reagents. After addition of potassium-1,4-naphthoquinone-2-sulfonate (Eastman Kodak Co.) in 0.01 M sodium phosphate, 0.144 M NaCl, 10^{-4} M EDTA, pH 7.4, or equivalent volume of buffer to the red cell suspension, resulting in a concentration of $2 \cdot 10^{-6}$ M naphthoquinone sulfonate, superoxide dismutase and catalase activities were determined on aliquots of cells taken at given intervals for a period of 2 h. All spectrophotometric measurements were performed on a Cary 14. Lysate catalase activity was ascertained by recording a decrease of hydrogen peroxide absorption at 240 nm, as described by Beers and Sizer [9]. Superoxide dismutase function of lysates was determined by a modification of a method of Heikkila, et al. [10] which assays superoxide dismutase by inhibition of the autooxidation of 6-hydroxydopamine (Aldrich Chemical Co., Inc.). Incubations of red cell suspensions were carried out as detailed above, in either the presence or absence of the catalase inhibitor 3-amino-1,2,4-triazole (Sigma Chemical Co.) [11,12] which was added following 15 min incubation in the presence or absence of 10^{-6} M naphthoquinone sulfonate.

Results

Control red cells demonstrated no alteration of catalase and superoxide dismutase activities for prolonged incubation periods (3 h), and were unaffected by either the presence or absence of glucose.

Without glucose in the medium, red cells incubated in the presence of $2 \cdot 10^{-6}$ M naphthoquinone sulfonate evidence an inhibition of catalase and superoxide dismutase activities with time, compared with red cell suspensions lacking naphthoquinone sulfonate. Catalase activity initially decreases more rapidly than superoxide dismutase, which is inhibited to a similar degree later in time (Fig. 1). The addition of ethanol to the suspension causes a partial reactivation of catalase activity.

Red cells incubated with the catalase inhibitor aminotriazole, in the presence

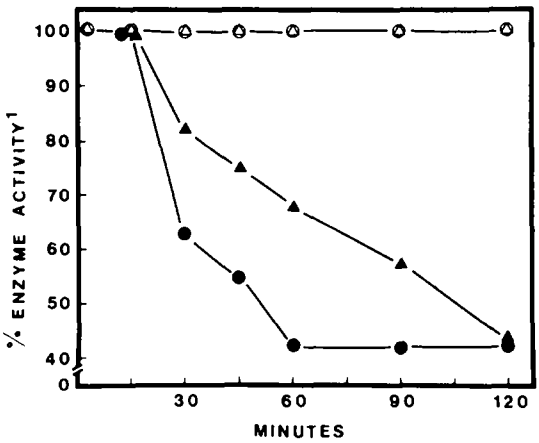

Fig. 1. Activity of catalase and superoxide dismutase in the presence of naphthoquinone sulfonate: 0.7% red cell suspensions in 0.01 M sodium phosphate buffer, 0.144 M NaCl, 10^{-4} M EDTA, pH 7.4, with or without 0.01 M glucose were incubated at 37°C for a period of 1.5 h. Naphthoquinone sulfonate was added to cell suspensions, with and without glucose, for a final concentration of $2 \cdot 10^{-6}$ M, and incubation of the cells at 37°C was continued. Aliquots of the cell suspensions were removed at time points indicated, and lysates were prepared by hypotonic lysis in distilled water followed by addition of 0.1 M sodium phosphate buffer, 10^{-4} M EDTA, pH 7.4. Catalase was assayed by further dilution of the lysate in 0.05 M sodium phosphate buffer, pH 6.8. Superoxide dismutase was assayed with further dilution of the lysate by introduction of 6-hydroxydopamine prepared in 0.001 M KCl/HCl, pH 2. ○, catalase, glucose present; ●, catalase, no glucose; △, superoxide dismutase, glucose present; ▲, superoxide dismutase, no glucose. Not depicted in the figure are control data indicating both catalase and superoxide dismutase activity remain unchanged during the entire incubation in the absence or presence of glucose. The data are representative of several assays done on separate days with similar differences in levels of activities.
[1] Percent enzyme activity relative to naphthoquinone sulfonate-free red cell lysate enzymes.

TABLE I

EFFECT OF AMINOTRIAZOLE ON RED CELL CATALASE AND SUPEROXIDE DISMUTASE IN PRESENCE OF NAPHTHOQUINONE SULFONATE

Conditions of incubation and assay: 0.7% red cell suspensions in 0.01 M sodium phosphate buffer, 0.144 M NaCl, 10^{-4} M EDTA, pH 7.4, with and without 0.01 M glucose were incubated at 37°C for a period of 1.5 h. Naphthoquinone sulfonate was added to the cell suspensions to a final concentration of 10^{-6} M and incubation at 37°C was continued for a period of 15 min at which time aminotriazole was added to cell suspensions with and without naphthoquinone sulfonate for a final concentration of 0.05 M aminotriazole. Incubation at 37°C continued for 45 min, at which time equal aliquots of all samples were removed and prepared for assay. Data are representative of several assays done on separate days with similar differences in levels of activity. Enzyme activity of lysate prepared after 60 min incubation of red cell suspension at 37°C using red cell suspension with no additions equal to 100.

Red cell suspension incubated in presence of:	Enzyme activity	
	Catalase	Superoxide dismutase
No additions	100	100
Aminotriazole	100	100
Glucose	100	100
Glucose + aminotriazole	100	100
10^{-6} M naphthoquinone sulfonate	77	82
10^{-6} M naphthoquinone sulfonate + aminotriazole	6	8
10^{-6} M naphthoquinone sulfonate + aminotriazole + glucose	14	17
10^{-6} M naphthoquinone sulfonate + glucose	100	100

TABLE II

EFFECT OF AMINOTRIAZOLE ON CATALASE AND SUPEROXIDE DISMUTASE IN PRESENCE OF NAPHTHOQUINONE SULFONATE IN DIFFERING CONCENTRATIONS

Conditions of incubation and assay same as in Table I. Enzyme activity of lysate prepared after 60 min incubation of red cell suspension at 37°C using red cell suspension with no additions equalling 100. Data are representative of several assays done on separate days with similar differences in levels of activity.

Red cell suspensions incubated with:	Enzyme activity	
	Catalase	Superoxide dismutase
No additions	100	100
+ aminotriazole	100	100
+ 10^{-6} M naphthoquinone sulfonate	76	80
$2 \cdot 10^{-6}$ M naphthoquinone sulfonate	43	68
10^{-6} M naphthoquinone sulfonate + aminotriazole	4	7
$2 \cdot 10^{-6}$ M naphthoquinone sulfonate + aminotriazole	0	0

or absence of glucose, demonstrate no inhibition of either catalase or superoxide dismutase activity in the absence of napthoquinone sulfonate. Red cell suspensions in the presence of aminotriazole, and treated with 10^{-6} M naphthoquinone sulfonate, show decreased activities of catalase and superoxide dismutase when compared to similarly treated cells in the absence of aminotriazole. It should be observed that glucose confers virtually no protection against naphthoquinone sulfonate-mediated enzyme inactivation in the red cell exposed to aminotriazole, unlike the protection evidenced in the absence of the catalase inhibitor (Table I).

The degree of catalase and superoxide dismutase inhibition is dependent on the napthoquinone sulfonate concentration. It is significant to note that the degree of inhibition of enzyme activities is markedly increased with naphthoquinone sulfonate and aminotriazole, over that seen with a 2-fold higher naphthoquinone sulfonate concentration without aminotriazole (Table II).

Assays of catalase and superoxide dismutase were performed following red cell suspension incubation at 37°C for 60 min in the presence of $2 \cdot 10^{-6}$ M naphthoquinone sulfonate in the presence of one of the following: glucose, 10^{-2} M; mannitol, 10^{-2} M; superoxide dismutase, 10 μg/ml and catalase, 3181 units/ml. Only the addition of glucose prevented loss of enzymatic activity.

Discussion

For many years hydrogen peroxide has been assumed to be a major mediator of the toxic effects of hemolytic drugs in the red cell. Hydrogen peroxide has been detected both in vitro [13,14] and in vivo [15] in red cells exposed to hemolytic agents. Introduction of hydrogen peroxide to red cells in vitro results in increased levels of methemoglobin, Heinz bodies and increased osmotic fragility [3,4,16] after normal cellular defense mechanisms against hydrogen peroxide have been exhausted, or, as in certain red cell enzyme deficiencies, normal defense mechanisms do not function [2,3].

Recent examinations of some hemolytic agents known to cause increased cellular levels of hydrogen peroxide have determined that an oxygen radical, superoxide anion, can be detected when such agents are in solution with adult human hemoglobin [6,17,18]. The toxicity of this radical toward the red cell is evident when superoxide anion is generated external to the cell by the auto-oxidation of dihydroxyfumaric acid [19]. A possible pharmacologic role of red cell superoxide dismustase has been demonstrated by intracellular inhibition of superoxide dismustase and subsequent exposure to naphthoquinone sulfonate [6].

It has been shown that hydrogen peroxide will cause the reduction and inactivation of a purified enzyme preparation of superoxide dismustase [7,8]. This led us to postulate that superoxide dismutase may become inactivated in the presence of a hemolytic agent such as naphthoquinone sulfonate if the cellular defense mechanisms against hydrogen peroxide were diminished. These studies have determined this to be the case. Naphthoquinone sulfonate-exposed red cells, in the absence of glucose, exhibit decreased catalase activity, probably through the formation of an inactive derivative, compound II [20—22], which has been detected in the presence of naphthoquinone sulfonate and other hemolytic agents [13—15]. Compound II is a functionally inactive form of catalase which may be reactivated in vitro by ethanol [20,21], and NADPH [23].

Diminished superoxide dismutase activity was observed with the decrease of catalase activity in a glucose-free medium whereas catalase activity, along with superoxide dismutase activity, is maintained in the presence of glucose in the normal red cell. A possible explanation for the latter finding is that glucose provides the red cell with another defense mechanism against hydrogen peroxide, through the formation of NADPH, a necessary component in the reduction of oxidized glutathione and possibly in the reduction of catalase compound II [23].

The effectiveness of aminotriazole as a catalase inhibitor has indicated that in the presence of naphthoquinone sulfonate, compound I of catalase has formed [11,12]. Compound I is an active complex of catalase and hydrogen peroxide and is a necessary component of the irreversible catalase-aminotriazole complex, which is completely inactive. Although glucose was present in naphthoquinone sulfonate solutions, catalase was markedly inactivated in the presence of aminotriazole, and superoxide dismutase closely followed suit. The absence of glucose in naphthoquinone sulfonate solutions containing aminotriazole resulted in almost the same enzyme inactivations, but with a consistently greater inactivation of both catalase and superoxide dismutase than that observed in the presence of glucose.

Inactivation of cellular catalase and superoxide dismutase is dependent on the naphthoquinone sulfonate concentration. Experiments where cells were incubated with both napthoquinone sulfonate and aminotriazole suggested the importance of catalase in the protection of superoxide dismutase, since inactivation of the cellular superoxide dismutase by incubation in a medium containing aminotriazole and naphthoquinone sulfonate exceeded the level of inactivation in a medium with twice the concentration of naphthoquinone sulfonate, but with no aminotriazole. Thus, the marked augmentation of super-

oxide dismutase inactivation in the presence of aminotriazole would seem to indicate that, under circumstance of these studies, the role of catalase is a pharmacologically important one in the protection of superoxide dismutase activity in a cell presumed to have a functional hexose monophosphate shunt.

In conclusion, we have shown that the presence of a hemolytic agent will cause an inactivation of catalase and superoxide dismutase, with glucose conferring protection against such inactivation. Further, it has been shown that catalase, under the circumstances described, provides protection of superoxide dismutase.

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