Erythrocyte Catalase

A Somatic Oxidant Defense?

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Abstract

Mammalian erythrocytes have large amounts of catalase, an enzyme which catabolizes hydrogen peroxide (H_2O_2). Because catalase has a low affinity for H_2O_2, others have suggested that glutathione peroxidase clears most H_2O_2 within the erythrocyte and that catalase is of little import. We hypothesized that erythrocyte catalase might function to protect heterologous somatic cells against challenge by high levels of exogenous H_2O_2 (e.g., in areas of inflammation). We find that, whereas nucleated cells (L1210 murine leukemia) are readily killed by an enzymatically generated flux of superoxide (and, therefore, H_2O_2), the addition of human and murine erythrocytes blocks lethal damage to the target cells. Inhibition of erythrocyte superoxide dismutase, depletion of glutathione, and lysis of the erythrocytes do not diminish this protection. However, inhibition of erythrocyte catalase abrogates the protective effect and the addition of purified catalase (but not superoxide dismutase) restores it. Furthermore, erythrocytes derived from congenitally hypocatalasemic mice (in which other antioxidant systems are intact) do not protect L1210 cells. Our results raise the possibility that the erythrocyte may serve as protection against by-products of its own cargo, oxygen.

Introduction

Mammalian erythrocytes are endowed with extraordinarily high activities of catalase (E.C. 1.11.1.6). Catalase decomposes hydrogen peroxide (H_2O_2) to water and oxygen either in a two-step catalatic reaction with H_2O_2 or in a peroxidatic reaction involving H_2O_2 and electron donors such as reduced pyridine nucleotides or ethanol (1).

Despite the remarkably high activity of catalase in erythrocytes, the function of this enzyme is in question. Catalase has a much lower affinity for H_2O_2 than does glutathione peroxidase. Indeed, others have suggested that, under physiological conditions, practically all H_2O_2 encountered by erythrocytes is detoxified by glutathione peroxidase and that catalase has no role in the clearance of H_2O_2 (2). Furthermore, humans lacking erythrocyte catalase activity are not even susceptible to hemolysis induced by oxidant drugs (3).

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Thus, mammalian erythrocytes have very high levels of an enzyme with no apparent function. In an attempt to clarify this situation, we have investigated the possibility that erythrocyte catalase functions as a "sink" for exogenous (i.e., extraerythrocytic) H_2O_2.

Methods

Growth of L1210 cells. Murine L1210 (lymphocytic leukemia) cells were raised in RPMI 1640 supplemented with 10% fetal calf serum. Upon reaching a density of $\sim 10^7$/ml, the cells were washed twice and suspended in the growth medium to a final concentration of 10^5/ml.

Preparation and treatment of erythrocytes. Human and murine blood was collected in heparin and the erythrocytes washed three times in sterile Hanks' balanced salt solution (HBSS). Lysed erythrocytes were prepared by freezing and thawing three times. Erythrocyte-reduced glutathione (GSH) was depleted by incubation of the cells in 10% hematocrit for 60 min with 2 mM 1-chloro-2,4-dinitrobenzene in HBSS (4). Erythrocyte superoxide dismutase (SOD)[1] was inhibited by incubation of erythrocytes at 10% hematocrit for 2 h at 37°C with 50 mM diethyldithiocarbamate in HBSS (5). Erythrocyte catalase activity was inhibited by preincubation of erythrocytes, 10% hematocrit, at 37°C for 14 h with 50 mM 3-amino-1,2,4-triazole in HBSS. In all cases, erythrocytes were washed thrice in HBSS following exposure to these inhibitors. In additional experiments, erythrocytes from normal and congenitally hypocatalasemic C_3H mice (6) were employed.

Assays. Erythrocyte GSH content was determined as described earlier (7). Catalase activity was assessed by spectrophotometric determination of the decomposition of H_2O_2 (1). SOD was measured as previously reported (8).

Experimental procedure. Oxidant-induced damage to L1210 was assessed by the extent of suppression of [^{3}H]thymidine incorporation. L1210 cells (10^5/ml) were incubated in RPMI 1640 with 10% fetal calf serum containing 0.1 μCi/ml [^{3}H]thymidine. Activated oxygen was generated by the addition of hypoxanthine (3.6 mM final concentration) and xanthine oxidase (100 mU/ml). By calculation, this should generate $\sim$0.1 μmol $\cdot O_2^-$/min. The extent of oxidant-induced damage to the target L1210 cells was estimated by the decrement in accumulation of acid-insoluble [^{3}H]thymidine, a reflection of nucleic acid synthesis. The cell suspensions (total incubation volume = 1.0 ml) were precipitated by the direct addition of 100 μl of 100% trichloroacetic acid. The precipitate was collected and washed on Whatman GF/C glass filters (Whatman Laboratory Products, Inc., Clifton, NJ). The precipitate was resolubilized by addition of these filters to toluene/Liquifluor/10% Biosolve and radioactivity determined by liquid scintillation counting.

Results

Under these experimental conditions, untreated L1210 cells accumulate [^{3}H]thymidine in a linear fashion over periods of more

1. *Abbreviations used in this paper:* SOD, superoxide dismutase.

Table I. Protection of L1210 against Oxidant Challenge by Variously Treated Human and Murine Erythrocytes

Treatment*	No.‡	[^{3}H]Thymidine uptake
		% of control±SD
A. HX	15	100
B. HX/XO	15	19 (±10.7)§
C. HX/XO + 7×10^7 human erythrocytes	9	95 (±35.1)
D. HX/XO + 7×10^7 human erythrocytes (lysed)	4	130 (±13.4)
E. HX/XO + 7×10^7 murine erythrocytes (normal)	4	77 (±19.8)
F. HX/XO + 7×10^7 murine erythrocytes (acatalasemic)	12	18 (±11.9)‖

* Explanation of treatments: HX = L1210 + hypoxanthine (no xanthine oxidase added); HX/XO = L1210 + hypoxanthine and xanthine oxidase. See text for experimental details.
‡ Number of independent determinations.
§ Significant difference from *A*, *C*, and *D* at $P \leq 0.001$ (Student's *t* test, two-tailed).
‖ Significant difference from *A*, *C*, *D*, and *E* at $P \leq 0.001$.

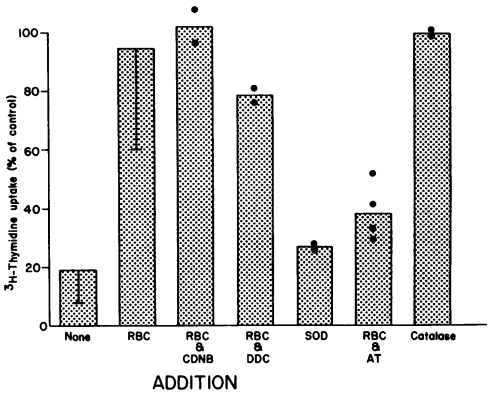

Figure 2. Protection of L1210 cells against oxidant challenge by variously treated erythrocytes and purified enzymes. In the absence of erythrocytes, [^{3}H]thymidine uptake is diminished by ~80% by hypoxanthine/xanthine. This is prevented by the addition of 10^7 normal human erythrocytes (*RBC*). Addition of the same number of erythrocytes treated with either 1-chloro-2,4-dinitrobenzene (*CDNB*) to deplete GSH or diethyldithiocarbamate (*DDC*) to inhibit SOD had negligible effect on this protection. However, erythrocytes pretreated with 3-amino-1,2,4-triazole (*AT*) (catalase activity, ~8% of initial) were ineffective in protection of L1210 cells. In accord with this, added purified catalase (1 mg/ml), but not SOD (50 μg/ml), was protective. Solid circles represent results of triplicate determinations.

than 1 h. However, during 30-min exposure of L1210 cells to hypoxanthine/xanthine oxidase, the incorporation of [^{3}H]thymidine is suppressed to 19(±10.7)% of control values (see Table I). Furthermore, 80% of cells treated in this fashion fail to exclude trypan blue. Remarkably, the addition of as few as 7×10^7/ml human erythrocytes completely prevents the O_2^-/H_2O_2-dependent suppression of [^{3}H]thymidine incorporation (Fig. 1) and normalizes trypan blue exclusion (not shown).

A number of manipulations were carried out to determine which erythrocyte oxidant defense systems mediate this protection of L1210 against exogenous oxidants. First, erythrocytes were lysed by freezing and thawing three times before addition to the L1210 hypoxanthine/xanthine oxidase system. Such lysed erythrocytes are fully protective of the L1210 cells (Table I). Second, erythrocyte GSH was completely reacted with 1-chloro-2,4-dinitrobenzene. GSH-depleted cells are also fully effective in protecting L1210 cells against hypoxanthine/xanthine oxidase-generated oxidants (Fig. 2). Third, inhibition of erythrocyte SOD by diethyldithiocarbamate (residual enzyme activity, ≤5% of initial) has little influence on the protective effects of erythrocytes. However, inhibition of erythrocyte catalase by preincubation with 3-amino-1,2-aminotriazole (residual activity, ~8% of initial) abrogates this protection (Fig. 2).

The fact that inhibition of catalase is the single maneuver which suppresses the protection of L1210 cells by erythrocytes suggests that this enzyme is responsible for the majority of the effect. This conclusion is strengthened by two additional lines of experimental evidence. First, the addition of purified catalase, but not SOD, duplicates the protection afforded by erythrocytes (Fig. 2). Second, normal mouse (C_3H) erythrocytes have similar protective effects. However, erythrocytes derived from hypocatalasemic C_3H mice (catalase activity ≤ 5% of normal) are not protective (Table I).

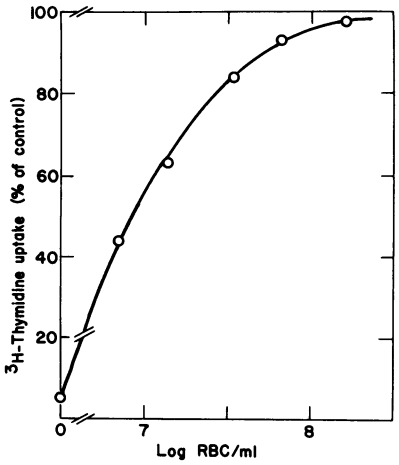

Figure 1. Protection of L1210 cells against hypoxanthine/xanthine oxidase-generated oxidants by human erythrocytes (*RBC*). Viability of L1210 cells was assessed by measurement of [^{3}H]thymidine incorporation. Individual points represent the mean of triplicate determinations. The results shown are derived from a single experiment.

Discussion

The marked protective effects, by added erythrocytes, of L1210 cells exposed to a flux of $\cdot O_2^-$ and H_2O_2 derived therefrom are in accord with observations recently published by two other groups. Toth et al. (9) found that perfusion of isolated rat lungs with $\cdot O_2$-generating systems or H_2O_2 caused vascular leakage and edema. The inclusion of erythrocytes in this model prevented such oxidant-induced damage. Furthermore, van Asbeck et al. (10) reported that prior insufflation of erythrocytes into the lungs

of rats protected the animals against early death due to oxygen toxicity.

Neither of these groups adduced conclusive evidence as to the erythrocyte oxidant defense system(s) involved in such protection. In the present investigations, we have employed a more reductionistic experimental design which allowed us to dissect the erythrocyte oxidant defense mechanisms. It appears likely that erythrocyte metabolism, GSH/glutathione peroxidase, and SOD are not important in protecting L1210 cells against oxidant challenge because lysis of the erythrocytes, depletion of GSH, and inhibition of SOD have no influence. By contrast, human or murine erythrocytes deficient in catalase activity are inefficient in staving off oxidant challenge to heterologous nucleated cells. Furthermore, the protection afforded L1210 cells by added erythrocytes is reproduced by the addition of purified catalase but not SOD.

We conclude that, under our experimental conditions, erythrocyte catalase protects heterologous somatic cells against exogenous oxidant challenge. The importance of the various erythrocyte anti-oxidant systems in protecting other cells against damage may well depend on the experimental or pathological system under study. In our conditions, there is a relatively brisk generation of $\cdot O_2^-$ and, therefore, H_2O_2. In other circumstances, steady state concentrations of H_2O_2 may be much lower, which favors participation of the GSH/glutathione peroxidase system with its higher affinity for substrate (2). However, in some situations (such as acute inflammation), erythrocyte catalase activity may be important in moderating the extent of inflammation and the oxidant damage to cells which are "innocent bystanders." Indeed, hypocatalasemic Japanese have severe oral gangrene (11) which may be attributable, in part, to the lack of erythrocyte antioxidant capacity and unregulated spread of inflammation. Our results may help explain the very high catalase activity in mammalian erythrocytes and suggest a reason for the extreme rarity of congenital hypocatalasemia in human populations.

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