Hydrogen Peroxide Utilization in Myeloperoxidase–Deficient Leukocytes: a Possible Microbicidal Control Mechanism

SEYMOUR J. KLEBANOFF and STEPHANIE H. PINCUS

From the U. S. Public Health Service Hospital, Seattle, Washington 98114, and the Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98195

ABSTRACT Phagocytosis-induced formate and glucose C-1 oxidation by the polymorphonuclear leukocytes of a patient with hereditary myeloperoxidase deficiency was considerably greater than normal. The addition of catalase to the leukocyte suspension was required for optimum formate oxidation. Azide and cyanide increased glucose C-1 oxidation by normal leukocytes but had little or no effect on myeloperoxidase-deficient leukocytes suggesting that these agents normally stimulate glucose C-1 oxidation, in part, by inhibition of myeloperoxidase. It is suggested that the inhibition or absence of myeloperoxidase results in an increased utilization of H_2O_2 in nonmyeloperoxidase-mediated H_2O_2-dependent reactions such as formate oxidation and hexose monophosphate pathway activation. The possibility of a microbicidal control mechanism in which a decrease in the microbicidal activity of myeloperoxidase is offset, in part, by an increase in the nonenzymatic microbicidal activity of H_2O_2 is considered.

INTRODUCTION

A massive and varied attack is mounted by the polymorphonuclear leukocyte (PMN)[1] against ingested microorganisms (for review see reference 1). It is probable that among the intraleukocytic microbicidal systems is one which consists of myeloperoxidase (MPO), H_2O_2, and an appropriate oxidizable cofactor such as a halide. What is the contribution of this system to the microbicidal activity of the cell? Patients with hereditary MPO deficiency are relatively free of infection. Of the five patients described, one had systemic candidiasis (2), whereas the remainder were in good health. Their leukocytes had decreased fungicidal and bactericidal activity (2, 3) although the defect was not as severe as in chronic granulomatous disease (CGD). This suggests several possibilities: (*a*) MPO-mediated antimicrobial systems play a relatively minor role in the intact PMN; (*b*) the level and variety of the intraleukocytic antimicrobial systems endow the PMN with an overkill capacity which allows it to function adequately under most circumstances despite the decrease or loss of one or other of the antimicrobial systems; (*c*) the loss of MPO is associated with an increase in the non-MPO–mediated antimicrobial systems of the cell. Evidence for the latter comes from studies with the use of the hemeprotein inhibitor, azide.

Azide greatly inhibited the microbicidal activity of normal PMN but had no effect on the microbicidal activity of MPO-deficient leukocytes (3). The antimicrobial systems of normal leukocytes can therefore be divided into two categories: azide sensitive (largely MPO-mediated) and azide insensitive (largely non-MPO–mediated). The extent of the inhibition by azide suggested that MPO-mediated systems contributed significantly to the microbicidal activity of the normal cell. Further, the azide-insensitive antimicrobial systems may be more active in MPO-deficient than in normal cells

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[1] *Abbreviations used in this paper:* CGD, chronic granulomatous disease; HMP, hexose monophosphate pathway; MPO, myeloperoxidase; PMN, polymorphonuclear leukocyte.

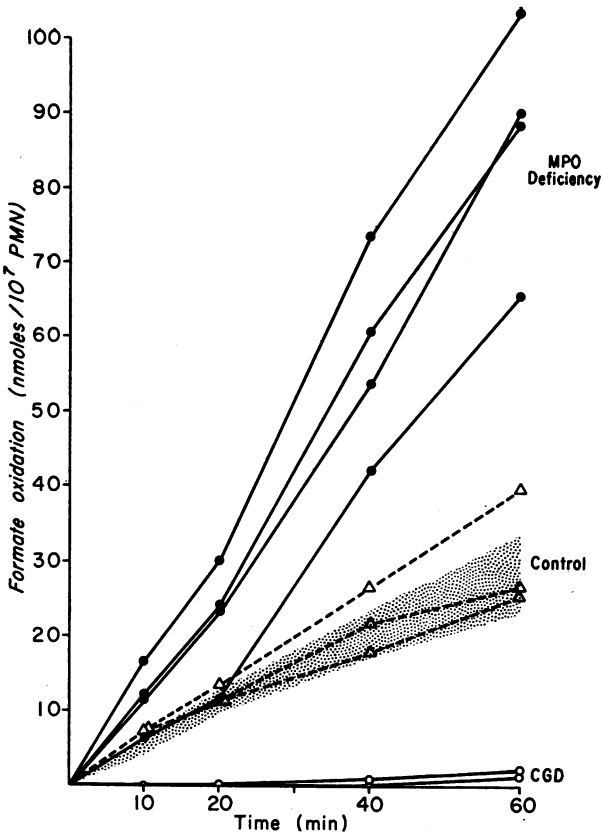

FIGURE 1 Formate oxidation by isolated leukocytes of one patient with hereditary MPO deficiency (●——●), two patients with chronic granulomatous disease (○——○), three patients with maturity-onset diabetes (△----△), and normal controls. The normal range (stippled area) is the 95% confidence interval (mean ±1.96 SEM). Each line is a separate experiment, and each point is the mean of duplicate values.

since the microbicidal activity of MPO-deficient leukocytes was greater than that of normal leukocytes in the presence of azide (3). H_2O_2 has been reported by Zatti, Rossi, and Patriarca (4) to accumulate in guinea pig PMN when utilization by hemeproteins is decreased by cyanide. The studies reported here indicate that in the absence of MPO, more H_2O_2 is available for non-MPO-mediated H_2O_2-dependent reactions. The possibility that an increase in the nonenzymatic microbicidal activity of H_2O_2 may occur in MPO-deficient leukocytes is therefore considered.

METHODS

Leukocytes were isolated from heparinized human venous blood as previously described (5). Blood was obtained from normal volunteers, from two male patients (D. C. and R. C.) with CGD, from one patient (C. B.) with hereditary MPO deficiency (2), and from male patients with maturity-onset diabetes.

Formate oxidation. The standard reaction mixture consisted of 2×10^7 PMN, 1000 nmoles of sodium formate-^{14}C (0.15 μCi), 1000 nmoles of glucose, 0.03 ml of a 10% suspension of latex particles (1.1 μ diameter, Dow Chemical Co., Midland, Mich.), 80 μg of catalase (Sigma Chemical Co., St. Louis, Mo.), 0.3 ml of calcium-free Krebs-Ringer phosphate buffer, pH 7.4, and water to a final volume of 0.5 ml.

Glucose C-1 oxidation. The standard reaction mixture consisted of 1×10^7 PMN, 1000 nmoles of glucose-1-^{14}C (0.05 μCi), 0.03 ml of a 10% suspension of latex particles (1.1 μ diameter), 0.3 ml of calcium-free Krebs-Ringer phosphate buffer, pH 7.4, and water to a final volume of 0.5 ml. Conversion of formate and glucose to $^{14}CO_2$ was determined as previously described (6) and expressed as nanomoles per 10^7 PMN.

RESULTS

H_2O_2 formed in intact PMN can be utilized for the conversion of formate-^{14}C to $^{14}CO_2$ in the presence of catalase (7), and this reaction has been employed for the detection of H_2O_2 in the intact cell. Fig. 1 and Table I confirm the marked reduction in formate oxidation by CGD leukocytes (8, 9) and demonstrate a striking increase in formate oxidation by MPO-deficient leukocytes ($P < 0.001$). The patient with hereditary MPO deficiency also had maturity-onset diabetes. Of three patients with maturity-onset diabetes, matched with respect to age, sex, and severity of diabetes, two fell within the normal range, and one was slightly elevated. Thus the marked increase in formate oxidation by MPO-

TABLE I
Effect of Catalase and Latex on Formate Oxidation

Supplements	Formate oxidation		
	Normal	MPO deficient	CGD
	nmoles/10⁷ PMN per hr		
Complete system	28.4 ±9.3 (18)	79.8 ±19.0 (6)	1.9 ±0.5 (4)
Complete system—catalase	13.4 ±2.9 (18)	30.0 ±5.6 (5)	0.8 ±0.9 (4)
Complete system—latex	3.6 ±1.9 (14)	6.0 ±3.3 (6)	0 ±0 (4)
Complete system—catalase and latex	1.5 ±1.2 (12)	3.8 ±1.9 (4)	0 ±0 (4)

The values are the mean ±SD; number of experimental values (n) in parenthesis; incubation 60 min.

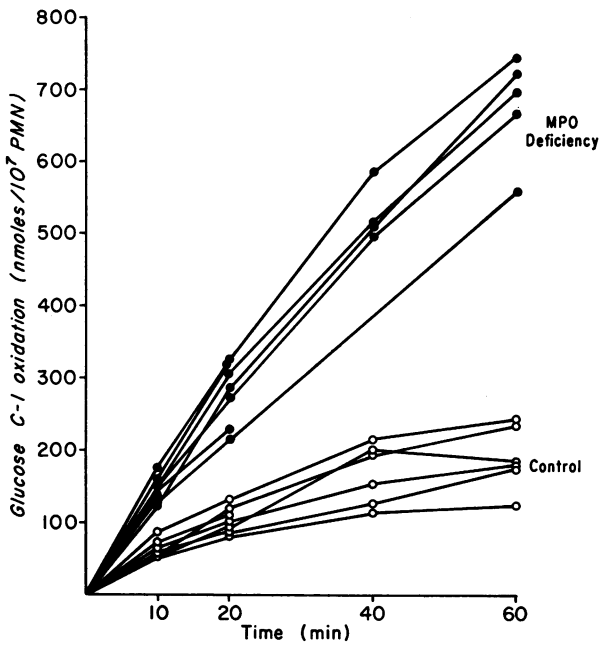

FIGURE 2 Glucose C-1 oxidation by isolated leukocytes from one patient with hereditary MPO deficiency (●——●) and normal controls (○——○). Each line is a separate experiment, and each point is the mean of duplicate values.

deficient leukocytes could not be attributed to diabetes. Formate oxidation by normal and MPO-deficient leukocytes was approximately doubled by the addition of catalase ($P < 0.001$) (Table I). This may be due to the passage of catalase into the cell with the particle and/or to the reaction of catalase with H_2O_2 which leaked into the extracellular fluid. The addition of catalase is thus recommended for optimum formate oxidation although it should be emphasized that, even in the presence of added catalase, formate oxidation may measure only a proportion of the H_2O_2 formed. When catalase is not added, formate oxidation is dependent upon endogenous catalase, and it is theoretically possible for a change in formate oxidation to reflect the availability of catalase rather than H_2O_2 formation. Formate oxidation by CGD leukocytes remained markedly reduced when catalase was added (Table I) suggesting that it is indeed a reflection of decreased H_2O_2 formation.

H_2O_2 formed in PMN also may be utilized by glutathione peroxidase for the oxidation of glutathione, which when coupled to the oxidation of NADPH by glutathione reductase, results in an increase in hexose monophosphate pathway (HMP) activity (10). Azide or cyanide increased glucose C-1 oxidation of rat PMN presumably through inhibition of hemeproteins (e.g. MPO, catalase) and the alternate utilization of H_2O_2 by the glutathione system (10). Azide and, at higher concentrations, cyanide stimulated glucose C-1 oxidation by normal leukocytes (Table II) suggesting that a similar reaction occurs in human cells. Azide had no effect, and cyanide had a greatly decreased effect on the glucose C-1 oxidation of MPO-deficient leukocytes, which suggests that these agents normally increase glucose C-1 oxidation, in part, by inhibition of MPO. Glucose C-1 oxidation by phagocytizing MPO-deficient leukocytes was considerably greater than that of normal cells ($P < 0.001$), whereas glucose C-1 oxidation by the leukocytes of the patients with maturity-onset diabetes was within the normal range under comparable conditions (Fig. 2).

DISCUSSION

In CGD, a decrease in leukocytic microbicidal activity is associated with a marked decrease in H_2O_2 formation as measured by formate oxidation and in HMP activity as measured by glucose C-1 oxidation (8). In contrast, in hereditary MPO deficiency, the leukocytic microbicidal defect is associated with an increase in both formate and glucose C-1 oxidation. This is due presumably to the decreased utilization of H_2O_2 by MPO in the MPO-deficient cells and its increased availability for other H_2O_2-dependent reactions i.e., oxidation of formate by catalase or stimulation of HMP activity via the glutathione system. The contribution of H_2O_2 deficiency to the microbicidal and metabolic defect in CGD is emphasized by the partial reversal of the lesion by the introduction of a H_2O_2-generating system into the cell (9, 11–13). In CGD one would expect a decrease in MPO-mediated *and* nonenzymatic H_2O_2-dependent antimicrobial systems, and the result is a profound microbicidal defect with severe disease. In hereditary MPO deficiency, although MPO-mediated antimicrobial systems are not operative, H_2O_2 is formed. The increased availability of H_2O_2 for non-MPO–catalyzed reactions in MPO-deficient leukocytes suggests that a decrease in the microbicidal activity of the peroxidase system may be offset in part by an increase in the nonenzymatic microbicidal activity of H_2O_2. This could account for the increase in the azide-insensitive microbicidal activity of

TABLE II

Effect of Azide and Cyanide on Glucose C-1 Oxidation

Supplement	Glucose C-1 oxidation	
	Normal	MPO deficient
	% stimulation	
Azide, 10^{-6} M	31.9 ($P < 0.001$)	3.3 (NS)
Cyanide, 10^{-3} M	55.0 ($P < 0.001$)	11.7 ($P < 0.05$)

n = 8; cells with azide or cyanide are compared with same cells without supplement using Student's t test for unpaired samples; incubation 20 min.

the MPO-deficient leukocytes and for the relative freedom from disease in this condition.

Is the microbicidal activity of H_2O_2 in the normal cell largely MPO-mediated or nonenzymatic? The results reported here suggest that MPO is responsible for the utilization of a significant amount of H_2O_2 in normal phagocytizing cells since in its absence, there is considerably more H_2O_2 available for non-MPO–catalyzed reactions. MPO increases the microbicidal activity of H_2O_2 many orders of magnitude, and certain inhibitors of peroxidase decrease the microbicidal activity of normal leukocytes (3). This suggests that the microbicidal activity of H_2O_2 in normal leukocytes may be, in large part, MPO mediated. The contribution of nonenzymatic H_2O_2-dependent microbicidal systems however may be inversely related to the availability of MPO for this reaction.

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