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Lethal oxidative damage to human immunodeficiency virus by human recombinant myeloperoxidase

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Human recombinant myeloperoxidase was evaluated in a cell-free system for its inactivation properties on the replication of human immunodeficiency virus, HTLV-III$_B$. In the presence of a hydrogen peroxide generating system (glucose and glucose oxidase) and sodium thiocyanate, the recombinant enzyme inhibited virus-induced syncytium formation and viral replication without causing any cytopathic effects on SupT1 reporter cells. In addition, U937 monocytoid cells, chronically infected with HIV1, were exposed to recombinant myeloperoxidase (10 U/ml) and monitored during 48 h for the accumulation of intracellular p24 viral antigen. Under these conditions, the recombinant enzyme significantly reduced intracellular viral replication without affecting cell viability.

Myeloperoxidase (recombinant); HIV; Viricidal activity; Inhibition of replication; Monocytic cell

1. INTRODUCTION

Inhibition of HIV infection by a variety of chemical drugs has been reported; in many cases, the therapeutical use of these compounds is hampered by toxic side effects [1]. The need for new antiviral agents, devoid of these problems, is more pressing than ever in view of the widespread occurrence of AIDS. Human polymorphonuclear leukocytes might be the source of such safe and efficient, albeit non-specific, agents. Indeed, neutrophils contain in their azurophilic granules an enzyme, myeloperoxidase, which displays strong inhibitory properties towards a large number of micro-organisms, including fungi and viruses [2–4]. In the presence of hydrogen peroxide, MPO catalyzes the oxidation of halides, or of a physiological pseudohalide such as thiocyanate [5], which in turn generates microbicidal compounds causing irreversible damage to the targets [6]. In mononuclear cells, production of MPO is restricted to fresh monocytes and is lost when these cells differentiate into resident tissue macrophages. These cells, however, still possess the hydrogen peroxidase generating system [7].

Macrophages are major in vivo reservoirs for human immuno deficiency viruses [8] and may be vectors for the spread of infection to different tissues. It is thus anticipated that any appropriate antiviral agent targetted to these infected cells may be able to prevent the dissemination of infectious particles. In this respect, MPO could be of interest since it has been shown to be internalized by macrophages in culture while maintaining its peroxidase activity [9].

MPO has usually been isolated from human polymorphonuclear cells or from the established HL60 promyelocytic cell line [10,11]. These sources, however, are inappropriate for large-scale preparation of the enzyme and have been replaced recently by engineered CHO cells overproducing recombinant myeloperoxidase [12]. Although this product corresponds to the unprocessed precursor of human myeloperoxidase, it displays biophysical and biochemical properties identical to those of the natural enzyme, including potent antibacterial activity [12]. In order to check if recombinant MPO also exhibits antiviral properties, we investigated its ability to inactivate the human immunodeficiency virus either in vitro when used in conjunction with the appropriate substrates or inside chronically infected U937 monocytoid cells in culture.

Abbreviations: MPO, myeloperoxidase (EC 1.11.1.7); recMPO, recombinant myeloperoxidase.

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2. MATERIALS AND METHODS

2.1. *Viricidal activity of recMPO in a cell-free system*

Inactivation assays proceeded as follows: an aliquot of a HTLV-III$_B$ viral suspension at 2.1×10^4 $TCID_{50}$/ml (60 μl), derived from persistently infected MOLT-3 cells [13], was incubated for 15 min at 37°C in the presence of glucose and glucose oxidase (the hydrogen peroxide generating system), sodium thiocyanate and recombinant MPO (0.8 U/μg [12]). These components, prepared as stock solutions in complete culture medium (RPMI medium, Gibco; 5% fetal calf serum, Seralab) were used either separately or in combination and at various concen-

trations. When necessary, volumes were adjusted to 500 μl with complete culture medium. Mixtures were then transferred onto SupT1 reporter cell pellets ($2 \cdot 10^6$; [14] and J. Hoxie, Univ. of Pennsylvania, USA) and further incubated for 45 min at 37°C. Cells were washed twice with complete culture medium, resuspended in 10 ml of fresh medium ($2 \cdot 10^5$ cells/ml) and allowed to grow at 37°C for up to ten days. Monitoring of cell cultures was done under the microscope at days 3, 5 and 7 post-infection in order to evaluate the cytopathic effect. On the same days and on day 10 post-infection, 0.5 ml of the cell suspensions was collected and frozen until use. Samples were then assayed for the p24 antigen using a murine monoclonal anti p24 antibody (NEN-Dupont, USA) and human anti-HIV immunoglobulins coupled to biotin. Complexes were detected with a streptavidin–horseradish peroxidase conjugate (Amersham) and the orthophenylene diamine chromogenic substrate (Sigma). Optical densities were recorded at 490 nm.

2.2. *Viricidal activity of recMPO on HIV1-infected U937 cells*

The human $CD4^+$ monocytoid cell line U937 (a gift from Dr. Sherman, Marseille, France) was maintained in RPMI medium supplemented with 10% fetal calf serum and 1% PSN (penicillin, streptomycin and neomycin antibiotic mixture, Gibco). The permanent ARV-4 cell line chronically producing HIV1 was used as a source of virus (a gift from Dr. J. Levy, San Francisco, USA). 10^6 U937 cells, prealably exposed to polybrene (2 μg/ml) for 30 min, were pelleted then incubated for 2 h in 1 ml of an ARV4 cell supernatant containing 160,000 pg of p24 antigen. Cells were washed twice with Hanks buffer (Gibco) and cultivated for three days. After washing, cells were exposed to recMPO at 10 U/ml medium and further cultivated for 48 h. Aliquots of the cells were collected at various times after addition of recMPO, washed three times, lyzed and assayed for the p24 antigen content, using the Abbott Laboratories antigen capture assay. Cell viability was assayed by the Trypan blue exclusion method.

3. RESULTS

3.1 *Viricidal activity of recMPO in a cell-free system*

Preliminary experiments consisted of testing if any of the separate components of the assay system could by themselves inhibit the development of the immunodefiency virus. In fact, neither glucose, glucose oxidase, sodium thiocyanate, nor recMPO had any significant effect on syncytium formation in virus-infected reporter cells and on viral replication (data not shown). Optimal concentrations of glucose oxidase, the enzymatic part of the H_2O_2 generating system, were then determined. It turned out that 60 mU/ml of the enzyme were generating excess hydrogen peroxide and were leading to cell death. Actual infection experiments were thus performed using either 0.6 or 1 mU/ml of glucose oxidase in the presence of 1% glucose and 20 μg/ml of NaSCN. Three recMPO concentrations, 8, 16 and 32 U/ml, were tested. As seen in Fig. 1 and Table I, in each case, virus-induced cytopathic effects, as seen under the microscope, and viral replication, as measured by a p24 antigen capture assay and expressed as variations in OD_{490}, were significantly reduced or totally suppressed. It appeared that glucose oxidase at 0.6 mU/ml did not generate enough H_2O_2 for full efficacy of recMPO whereas this was achieved with a tenfold increase in glucose oxidase. As expected, the effects of recMPO were strictly dependent on the simultaneous presence of hydrogen peroxide, as provided by the generating system, and of sodium thiocyanate.

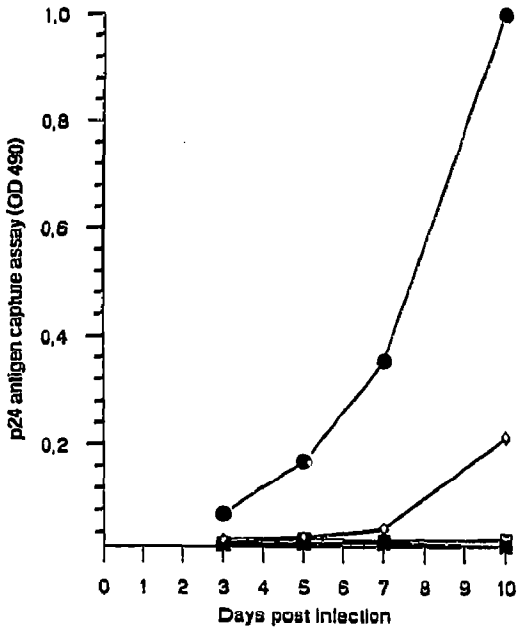

Fig. 1. Inhibition of $HTLV\text{-}III_B$ replication, in vitro, by human recMPO. The $HTLV\text{-}III_B$ virus (1200 T-cell infectious dosis 50%) was incubated with recMPO (8 U/ml) in the presence of sodium thiocyanate and a hydrogen peroxide generating system. The treated virus was then used to infect reporter SupT1 human cells. Growth of the virus in infected cells was monitored for ten days using a p24 antigen capture assay. Data are expressed in milliunits OD_{490}. (●), positive control, reporter cells infected with untreated virus; (□), negative control, uninfected reporter cells; (◇), reporter cells infected with the $HTLV\text{-}III_B$ virus treated with 8 U/ml of recMPO in the presence of 0.6 mU/ml of glucose oxidase, 1% glucose and 20 μg/ml NaSCN; (■), same conditions as above, except the concentration of glucose oxidase (6 mU/ml).

3.2. *Viricidal effect of recMPO on intracellular HIV1 replication*

Preliminary experiments consisted of testing the effect of recMPO on the growth of HIV1 infected U937 cells. Over a time span of 72 h, recMPO at a final concentration of 12 U/ml in the culture medium did not affect cell viability. Starting from $2 \cdot 10^5$ viable cells/ml, the culture reached 10^6 viable cells/ml after three days in the presence or absence of the recombinant enzyme.

The subsequent experiment was designed to evaluate the effect of recMPO on the intracellular replication of the virus. To this end, U937 cells infected with HIV1 were washed to remove the extracellular virus, then exposed to recMPO at 10 U/ml in culture medium and further incubated. Cells were collected at 2, 6, 20, 24 and 48 h after addition of recMPO, washed, tested again for viability, lyzed and assayed for the intracellular p24 antigen content. As seen in Fig. 2, viral replication in U937 cells was prevented by recMPO for at least 20 h. (Beyond that period, viral replication in untreated infected cells started to decline.)

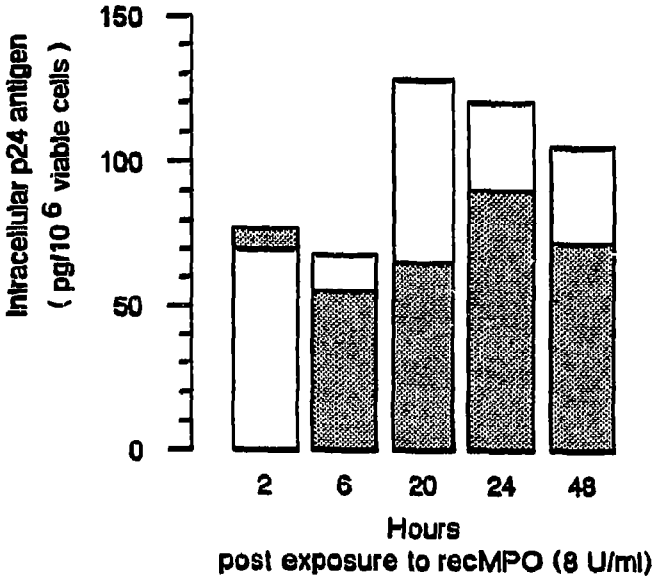

Fig. 2. Inhibition of viral replication by recMPO in chronically infected U937 cells. U937 monocytoid cells chronically infected with HIV1 (see section 2) were exposed to 10 U/ml of recMPO in the culture medium. Samples were collected at various times, lysed and assayed for p24 content. Data are expressed in pg p24 per 10^6 viable cells. (□), untreated infected U937 cells; (■), recMPO-treated infected U937 cells.

Table I

Antiviral activity of human recombinant myeloperoxidase in a cell-free system.

Assay		Monitoring	Days post infection			
			3	5	7	10
Controls						
T1	Supl Reporter cells,	CPE	(+)	+	++	nd
	+ virus	OD_{490}	55	144	331	975
T2	T1 + glucose	CPE	(+)	+	++	nd
	oxidase 0.6 mU/ml	OD_{490}	92	184	405	750
T3	T1 + glucose	CPE	(+)	+	++	nd
	oxidase 6 mU/ml	OD_{490}	97	368	635	775
Complete systems						
T4	Glucose oxidase 0.6 mU/ml	CPE	–	–	(+)	nd
	and recMPO	OD_{490}	7	3	22	189
	8 U/ml	% inhib.	92.4	98.4	94.5	74.8
T5	T4 with recMPO	CPE	–	–	(+)	nd
	16 U/ml	OD_{490}	12	0	29	328
		% inhib.	87	100	92.8	56.2
T6	T4 with recMPO	CPE	–	–	(+)	nd
	32 U/ml	OD_{490}	5	10	17	240
		% inhib.	94.5	94.5	95.8	68
T7–T9	Glucose oxidase	CPE	–	–	–	nd
	6 mU/ml	OD_{490}	0	0	0	0
	and recMPO at	% inhib.	100	100	100	100
	8, 16, or 32 U/ml					

CPE: cytopathic effects observed under the microscope; –: no syncytia; (+): a few syncytiae; + and ++: increase in size and number of syncytiae.
OD_{490}: optical densities in milliunits at 490 nm.
nd: not done.
Complete systems: indicates the presence, in the mixtures, of HTLV-III_B virus, SupT1 cells, glucose, glucose oxidase, sodium thiocyanate and recMPO.

4. DISCUSSION

The data show that human recombinant MPO, in the presence of appropriate substrates, irreversibly inactivates the HTLV-III_B virus in vitro, as was recently shown also for the natural enzyme [15]. In addition, recMPO appears to inhibit intracellular viral replication in U937 monocytoid cells in culture, chronically infected with HIV1. Lactoperoxidase-mediated killing of envelopped viruses has already been documented [16–18]. MPO, however, offers several advantages over other peroxidases as a potential in vivo therapeutic agent. As a natural human enzyme, it is expected to be non-immunogenic and non-toxic; in addition, it is now available on a large scale via engineered CHO cells in culture [12]. MPO functions naturally in the biological environment of monocytes, which provide the appropriate substances, NADPH, NADPH oxidase and halide ions, for optimal enzyme activity [2,3,5]. Macrophages, the differentiated form of monocytes, although lacking MPO activity, still present the same hydrogen peroxide generating system [7]. Specific targetting of recMPO to macrophages could thus restore a strong oxidative capability, which would help fighting off infections of these cells. In conclusion, the results presented here warrant further detailed studies on the role of recMPO as an efficient antiviral agent in vivo.

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