

Peroxidase in Therapy of Experimentally Induced Leprosy

TO THE EDITOR:

Although achievements have been made in leprosy therapy the need for new antileprosy agents remains. The bactericidal role of myeloperoxidase (MP) of neutrophilic granulocytes and cells of the monocyte-macrophage system is well known. In leprosy a level of MP activity in phagocytes represents the cell-mediated immune state, and MP estimation might be used for prognosis of the severity of leprosy infection⁽⁴⁾.

Through artificial decrease in MP activity of mouse peritoneal macrophages we could obtain prolonged phagocytic persistence of the agents of some chronic mycobacterial infections (*Mycobacterium tuberculosis* and *M. leprae*). Using electron microscopic methods of investigation, it was shown that mice with experimentally induced leprosy demonstrated more intense development and generalization of the disease (multiplication of *M. leprae* in lung and splenic tissues) against the background of decreased activity of phagocytic MP in the site of inoculation (foot pad)⁽⁵⁾.

In view of the significant role of phagocytic MP in leprosy pathogenesis, we tried to treat *M. leprae*-infected mice⁽⁸⁾ by ad-

ministering them lyophilized horseradish peroxidase (HP) with their diet *per os*.

The mice were infected into the right hind foot pads with *M. leprae* suspensions (at a dose of 10^4 per foot pad) taken from lepromatous (LL) leprosy patients and passed on mice one or two times. In order to choose the most effective antibacterial dose of the preparation test mice were given HP (Merk) activity equaling 100 U/mg at a dosage of 50, 100, 150, 200 and 250 mg per 1 kg of fodder mixture. Mice infected simultaneously with test animals and fed with 100 mg of diaminodiphenyl sulfone (DDS) per kg of food and untreated mice were used as controls. Drug trials were carried out continuously according to the World Health Organization (WHO) guide⁽¹⁰⁾. Five or six mice from each group were decapitated 5, 8 and 11 months after the experiments had been started.

MP activity in loaded *M. leprae* phagocytes was detected using an electron cytochemical method⁽²⁾. MP activity in neutrophilic granulocytes (NG) of peripheral blood and the hepatic functional state (as judged by aminotransferase activity) and blood counts were determined in mice using standard methods⁽⁷⁾ 5, 7, 9 and 11 months after beginning the experiment (at a

TABLE 1. Dynamics of *M. leprae* growth in mouse foot pads (10^6 *M. leprae*, $M \pm m$).

HP dose (mg/kg of food)	Mos. after infection	Untreated controls	Treatment by	
			DDS	HP
50	5	1.01 ± 0.26	0.31 ± 0.16	0.73 ± 0.16
	8	7.85 ± 2.93	2.73 ± 0.79	3.48 ± 0.72
	11	5.51 ± 1.11	2.48 ± 0.32 ^a	2.32 ± 0.27 ^a
100	5	4.2 ± 2.0	3.0 ± 1.5	2.8 ± 1.1
	8	285 ± 109	96.0 ± 18.4 ^a	19.9 ± 9.6 ^a
	11	186.2 ± 54.0	53.4 ± 14.1 ^a	9.7 ± 3.5 ^a
150	5	22.5 ± 5.27	9.28 ± 2.47	5.27 ± 1.71 ^b
	8	140.7 ± 32.9	41.92 ± 10.64 ^a	10.75 ± 3.09 ^c
	11	116.2 ± 32.8	31.54 ± 10.80 ^a	7.51 ± 2.02 ^b
200	5	17.51 ± 4.70	6.30 ± 1.04	3.25 ± 1.57 ^b
	8	88.2 ± 12.8	36.50 ± 9.13	7.02 ± 2.68 ^a
	11	61.25 ± 7.34	15.54 ± 5.65 ^a	3.50 ± 1.53 ^b
250	5	20.30 ± 5.18	4.08 ± 0.30 ^a	3.50 ± 0.71 ^a
	8	66.53 ± 10.70	37.94 ± 8.74	24.4 ± 6.3
	11	74.30 ± 7.12	24.1 ± 4.6	20.08 ± 5.23

^a p < 0.01 as compared with controls.

^b p < 0.02 as compared with controls.

^c p < 0.05 as compared with controls.

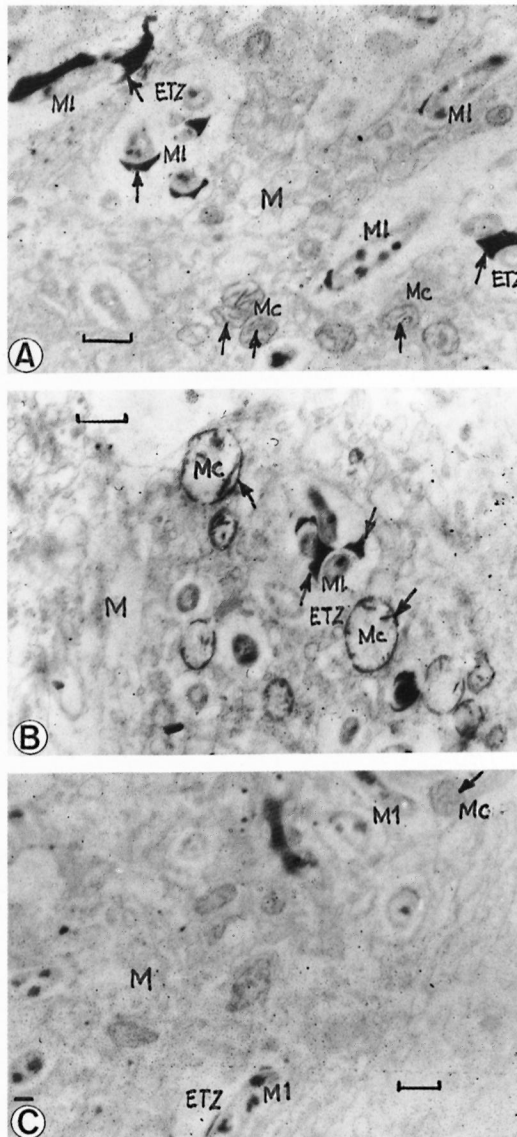


FIG. 1. Ultrathin sections of macrophages (M), loaded with *M. leprae* (MI). Localization of MP activity (arrows) in membranes and crystals of mitochondria (Mc) and electron-transparent zone (ETZ) around *M. leprae* against the background of treatment with HP (A), DDS (B) and without treatment (C) (no staining; bar = 0.5 μ m).

dosage of 150 mg/kg of food). *M. leprae* in the mouse foot pads were counted by the method of Shepard and McRae (⁹). As in the experiments *M. leprae* from different patients were used, and the intensity of their multiplication varied depending upon the strain. Within each group of mice the same strain of *M. leprae* was used.

When introducing HP at doses of 100, 150

and 200 mg/kg of the food, a strong delay of *M. leprae* growth was observed. After 5 months of treatment, the count of *M. leprae* in mice treated with HP was 1.5 to 5 and 1.4 to 3 times less compared with untreated and DDS-treated control animals, respectively (Table 1). After 8 months of treatment the antimicrobial effect of HP was more marked: *M. leprae* in HP-treated mice were 12 to 13 and 4 to 6 times less than in untreated and DDS-treated animals, respectively. After 11 months of treatment a tendency toward inhibition of *M. leprae* growth in mice treated with HP remained at the same level. Treatment with HP at 50 and 250 mg per 1 kg of the food was less effective (Table 1) (⁹).

Electron microscopy of ultrathin sections of infiltrated foot pads of mice showed electron-dense reactive products (markers of MP activity) in the cytoplasm of *M. leprae*-loaded macrophages on membranes and crystals of mitochondria, membranes of phagosomes and, mainly, in phagolysosomes in the vicinity of *M. leprae*. MP activity in phagolysosomes was higher when treating with HP and DDS (Fig. 1).

A cytochemical investigation of blood NG from mice treated with HP and DDS for 5 months showed a strong tendency to increase intracellular MP. By the end of 7 months of treatment, an increase in MP activity was significant and remained such until the end of the experiment. In addition, in all groups a significant increase in enzymatic activity was observed by month 11 of the experiment ($p < 0.05$). In untreated mice activity of endogenous MP decreased within the periods indicated ($t = 3.3$; $p < 0.05$) (Table 2).

TABLE 2. Levels of MP activity in peripheral blood neutrophils in mice at various times after infection and treatment.

Mos. after Infection	Average cytochemical coefficient of neutrophilic MPO		
	HP treatment	DDS treatment	No treatment
5	1.9 \pm 0.08	1.75 \pm 0.05	1.7 \pm 0.03
7	1.86 \pm 0.05 ^a	1.77 \pm 0.03 ^b	1.68 \pm 0.05
9	1.87 \pm 0.03 ^a	1.81 \pm 0.04 ^b	1.65 \pm 0.03
11	2.54 \pm 0.19 ^a	2.22 \pm 0.06 ^a	1.58 \pm 0.02

^a $p < 0.01$ as compared with controls (without treatment).

^b $p < 0.05$ as compared with controls (without treatment).

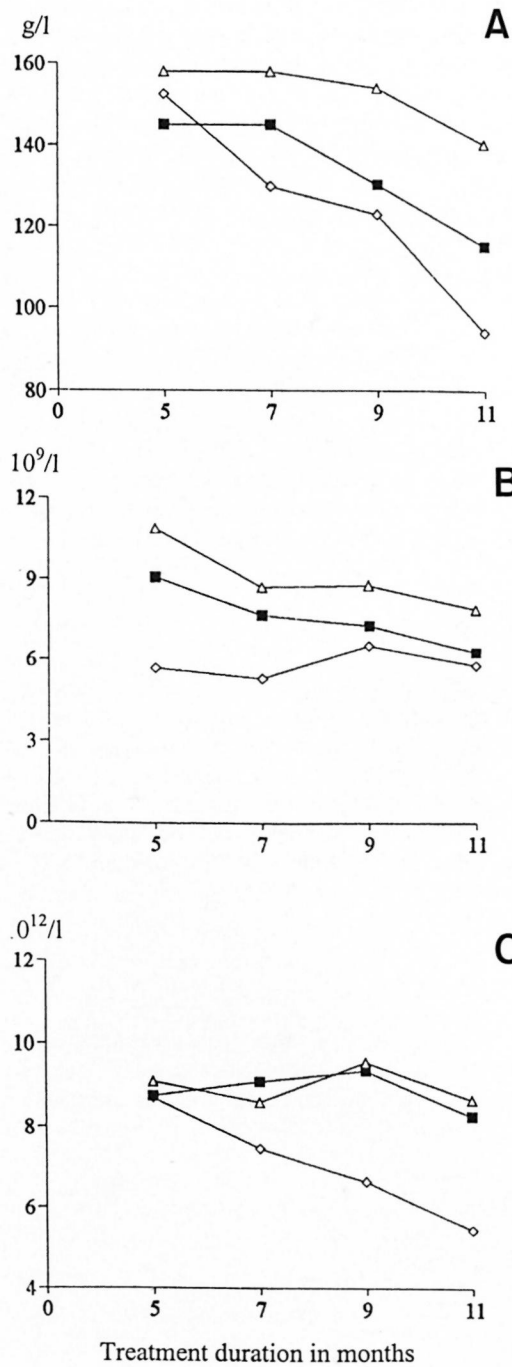


FIG. 2. Changes in hemoglobin level (A), number of leukocytes (B) and erythrocytes (C) in blood of mice. ◇ = Treatment with HP; ■ = treatment with DDS; △ = without treatment.

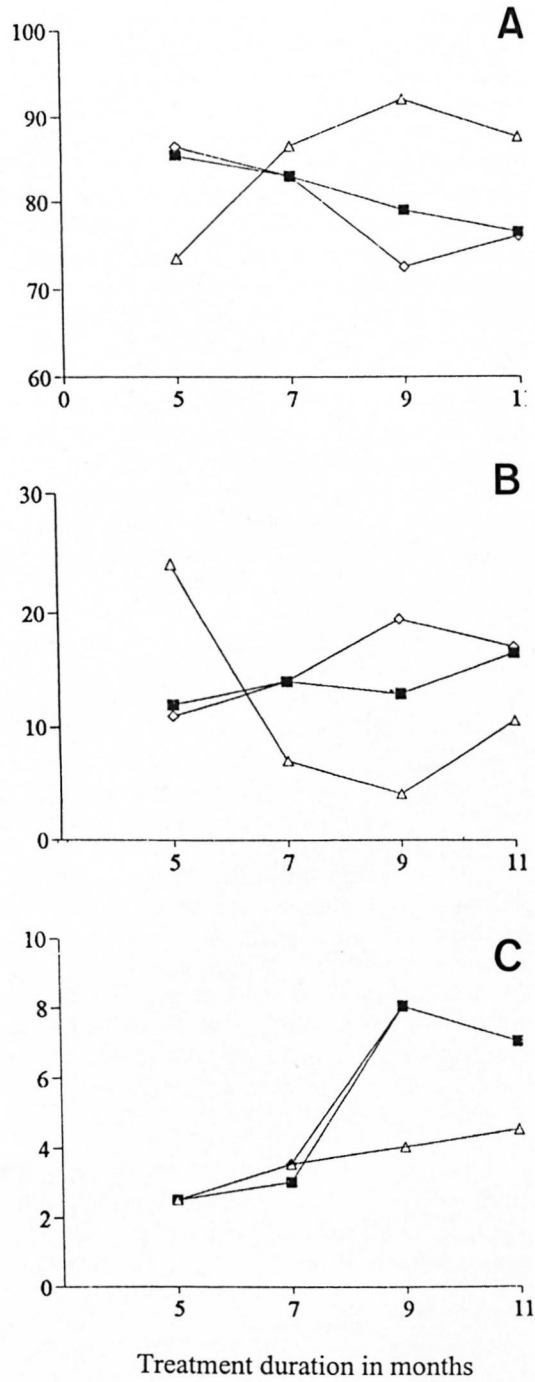


FIG. 3. Changes in percentage of lymphocytes (A), neutrophils (B) and monocytes (C) in blood of mice. ◇ = Treatment with HP; ■ = treatment with DDS; △ = without treatment.

Administration of HP to mice during months 7, 9 and 11 caused a significant decrease in the levels of hemoglobin and erythrocytes compared with the control animals, and activation of the anti-oxidative system might be one of the possible mechanisms (Fig. 2). In this connection, highly active antibacterial products of MP might have a cytopathic action on cellular membranes, causing their destruction (¹).

There were some changes on hemograms from mice administered HP and DDS. Leukocyte counts significantly decreased after 5 months of HP administration versus DDS-treated and untreated animals. The tendency continued during months 7, 9 and 11 of HP treatment, suggesting the decrease of inflammation caused by introducing the infectious agent (Fig. 2). By month 5 of the experiment, a significant increase in the percentage of lymphocytes was observed, while the number of neutrophils was decreased. Later in the experiment (months 9 and 11), the proportion of neutrophils in leukocytic counts was increased, while the percent of leukocytes was decreased. The monocytic fraction significantly increased, suggesting the ability of HP and DDS to stimulate phagocytic activity of the blood (⁶) (Fig. 3).

Aminotransferase activity in hepatic cells remained at the base-line level over the entire period of the experiment.

A mechanism of antibacterial activity of DDS has not yet been elucidated. The present investigation shows that DDS action is directed toward increasing the level of activity of phagocytic myeloperoxidase. This is true for HP. Moreover, DDS and HP promote stimulation of cell-mediated immunity. Thus, treatment with HP exerts a stimulating action by increasing intracellular phagocytic MP (possibly, at the expense of endogenous HP) and, hence, bactericidal activity.

The results obtained showed that clinical application of the enzyme has considerable

promise. High effectiveness of HP when administered *per os* permits the use of this enzyme as a biological supplement to food.

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