Effects of Peroxidase Therapy on Functional State of the Liver and Phagocytes and Blood Cell Counts in Mice with Experimental Leprosy

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We studied the effects of long-term therapy with horseradish peroxidase administered orally to mice with experimental leprosy. Horseradish peroxidase stimulated phagocyte myeloperoxidase activity that correlated with their functional activity, reduced leukocytosis, and produced no adverse effects on the liver.

Key Words: *experimental leprosy; peroxidase; activity of phagocytes; hemogram*

Despite a notable progress in the treatment of leprosy, elaboration of new antileprosy drugs is still a topical problem. Long-term therapy with antileprosy drugs produces side effects and induces drug resistance in *Micobacterium leprae* [4,6].

Our previous studied demonstrated that deficiency of the phagocytic myeloperoxidase system (the major antibacterial systems of neutrophilic granulocytes, monocytes, and macrophages) plays a role in the pathogenesis of leprosy [3]. Experiments on leprosy model designed by C. Shepard [8] and used for testing antileprosy preparations showed that lyophilized horseradish peroxidase (HP, 100 U/mg) and dried and grated roots of this plant administered orally in a dose of 100-200 mg/kg food produce strong antibacterial effects in mice. HP applied for 8-11 months inhibits the growth of *M. leprae* by 14-19 times compared to the control, and its efficiency 4-5-fold surpasses that of the major antileprosy drug 4,4'-diaminodiphenyl sulfone.

Here we studied the effects of HP on the state and activity of myeloperoxidase (MPO) in phagocytes of the inflammatory infiltrate and blood neutrophilic granulocytes (NG), total blood cell count, and functional state of the liver in mice (to exclude hepatotoxicity of HP in long-term administration).

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MATERIALS AND METHODS

Experiments were performed on 150 CBA mice kept under standard conditions. The mice were infected by intraplantar injection of *M. leprae* suspension (10^4 mycobacteria/foot) isolated from patients with lepromatous leprosy and once passed in experimental animals. Group 1 mice fed a diet containing lyophilized HP (Merck, 100 U/mg, 150 mg/kg food). Group 2 mice received 4,4'-diaminodiphenyl sulfone (100 mg/kg food). Untreated mice served as the control. These preparations were tested by the continuous method as recommended by the World Health Organization [7]. Five to 6 mice in each group were decapitated 5, 7, 9, and 11 months after the start of the experiments to obtain the blood, inflammatory infiltrate, and liver.

MPO activity in cells phagocytizing *M. leprae* was measured by the electron cytochemical method [5]. MPO activity in peripheral blood NG was estimated by a semiquantitative method proposed by Astaldi and Verga. The content of hemoglobin, erythrocytes, and leukocytes and white blood cell counts were estimated routinely. Activities of alanine and aspartate transaminases (ALT and AST) in the plasma and liver homogenates were measured as described elsewhere [2].

The results were analyzed by Student's *t* test (Statgraphics software).

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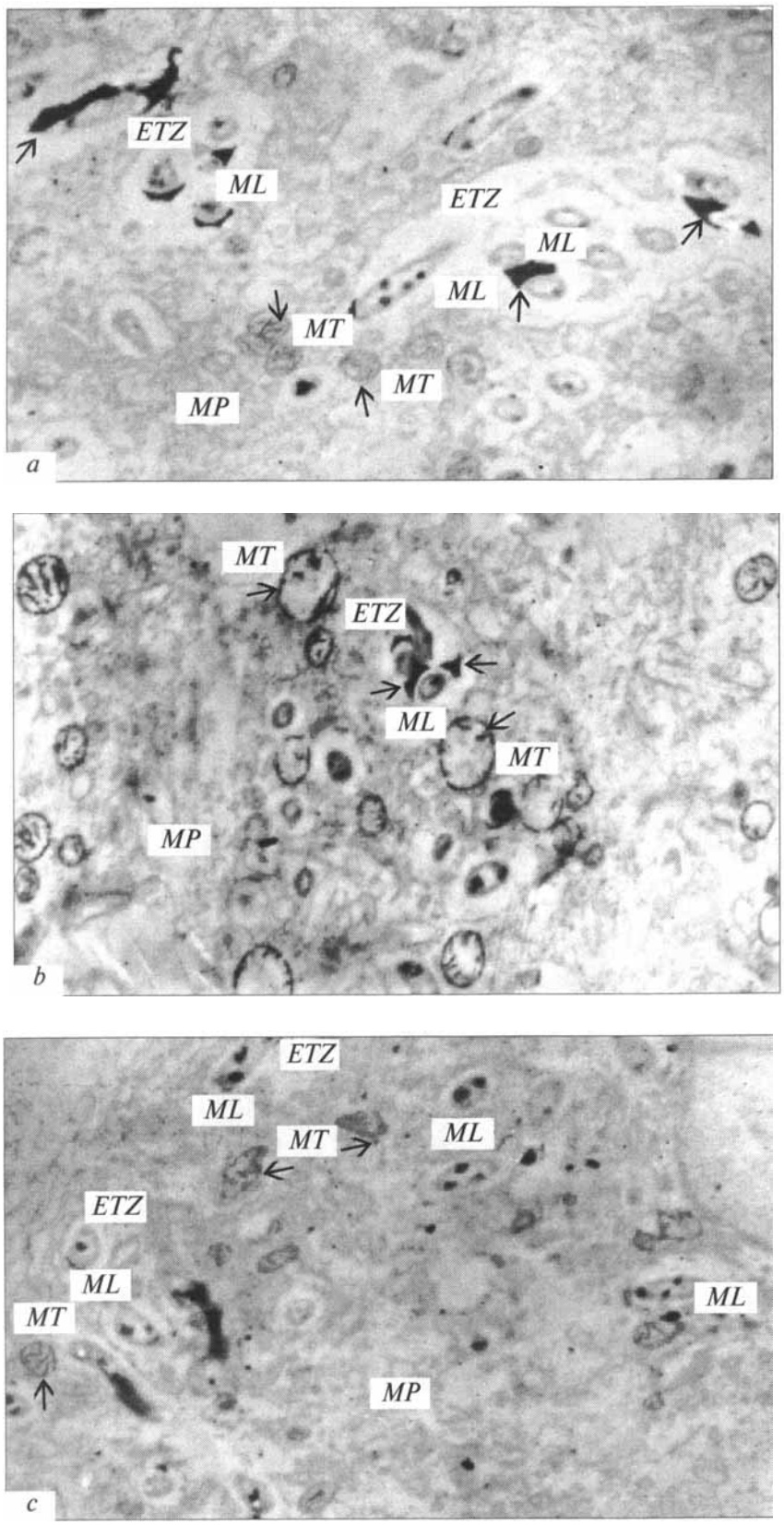

Fig. 1. Ultrathin sections of macrophages (MP) containing *Micobacterium leprae* (ML): therapy with horseradish peroxidase (*a*) or 4,4'-diaminodiphenyl sulfone (*b*) or without treatment (*c*). No staining, ×13 000. Arrows: myeloperoxidase activity in membranes and cristae of mitochondria (MT) and in the electron-transparent zone (ETZ) around *M. leprae*.

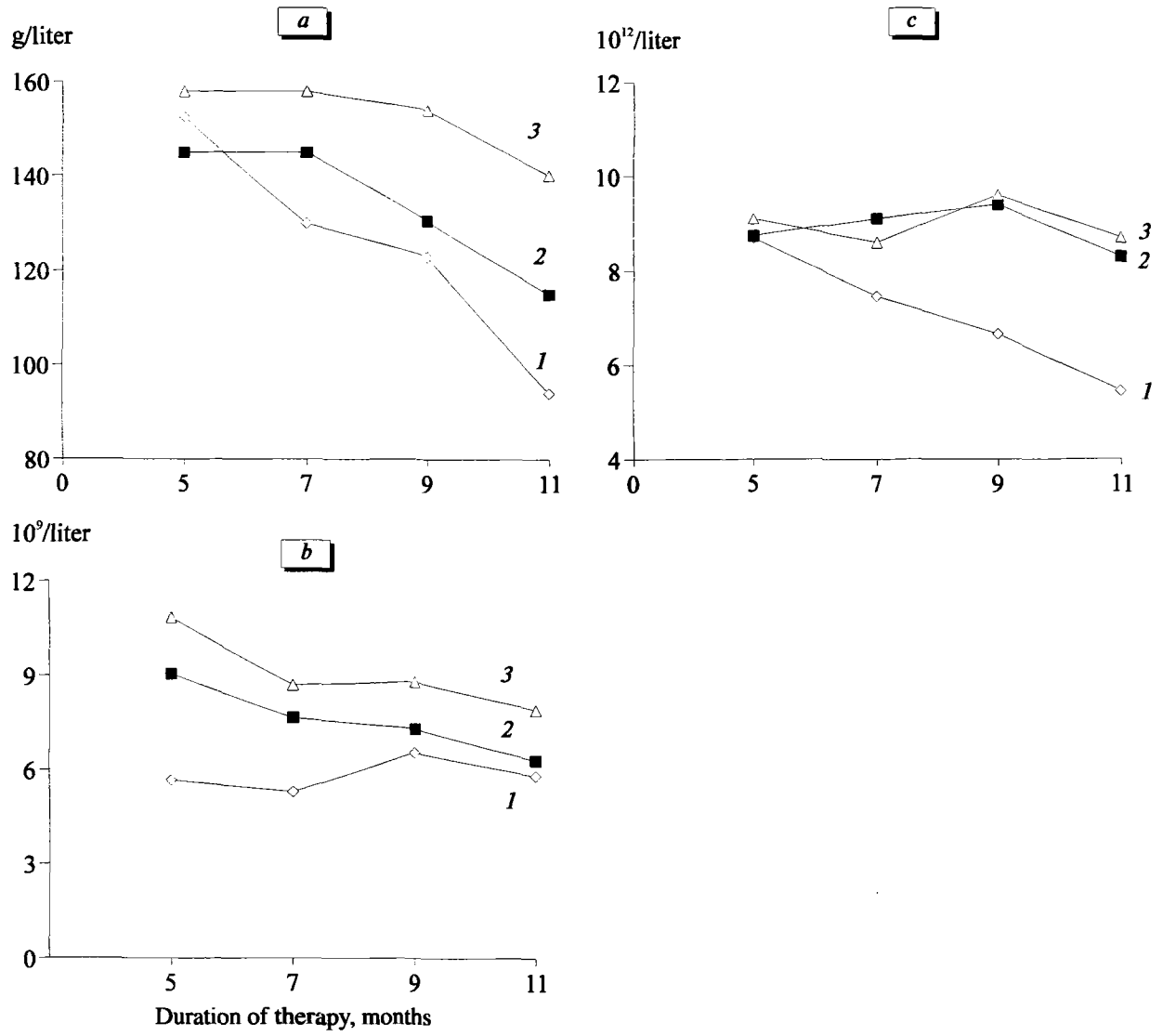

Fig. 2. Hemoglobin content (*a*) and leukocyte (*b*) and erythrocytes (*c*) counts in mouse blood. Here and in Fig. 3: therapy with horseradish peroxidase (*1*) or 4,4'-diaminodiphenyl sulfone (*2*) or without treatment (*3*).

RESULTS

Electron microscopy of ultrathin slices of pad inflammatory infiltrates revealed MPO activity in mitochondrial membranes and cristae and in electron-transparent zone around *M. leprae* in macrophage cytoplasm. By contrast to control animals, these deposits were prevalent in groups 1 and 2 mice indicating a higher activity of MPO (Fig. 1).

Cytochemical studies showed that activity of intracellular MPO in peripheral blood NG tended to increase in groups 1 and 2 mice 5 months after the start of therapy. This elevation was statistically significant from the 7th month of treatment to the end of observations. In addition, MPO activity increased by the 11th month of therapy ($p<0.05$), whereas in control mice this parameter decreased ($t=3.3$, $p<0.05$, Table 1).

HP therapy for 7, 9, and 11 months considerably decreased the content of hemoglobin and erythrocyte count compared to the control. In group 2 mice, hemoglobin content also decreased, while erythrocyte count remained unchanged.

These changes observed after long-term treatment with HP probably attest to considerable activation of the antioxidant system in cells. Highly active antibacterial MPO products can produce cytopathic effects and cause destruction of the plasma membrane [1], thus leading to erythrocytolysis, anemia, and erythropenia. Long-term 4,4'-diaminodiphenyl sulfone therapy of *M. leprae*-infected patients also causes side effects, in particular persistent anemia [4].

Leukocyte count in group 1 mice treated with HP for 5 months considerably decreased compared to the control and group 2 animals. These differences persis-

TABLE 1. MPO Activity (Rel. U) in Peripheral Blood Neutrophils in Mice ($M \pm m$)

Time after inoculation, months	Control (without therapy)	Therapy	
		HP	4,4'-diaminodiphenyl sulfone
5	1.70±0.03	1.90±0.08	1.75±0.05
7	1.68±0.05	1.86±0.05*	1.77±0.03**
9	1.65±0.03	1.87±0.03*	1.81±0.04**
11	1.58±0.02	2.54±0.19*	2.22±0.06*

Note. *$p<0.01$ and **$p<0.05$ compared to the control.

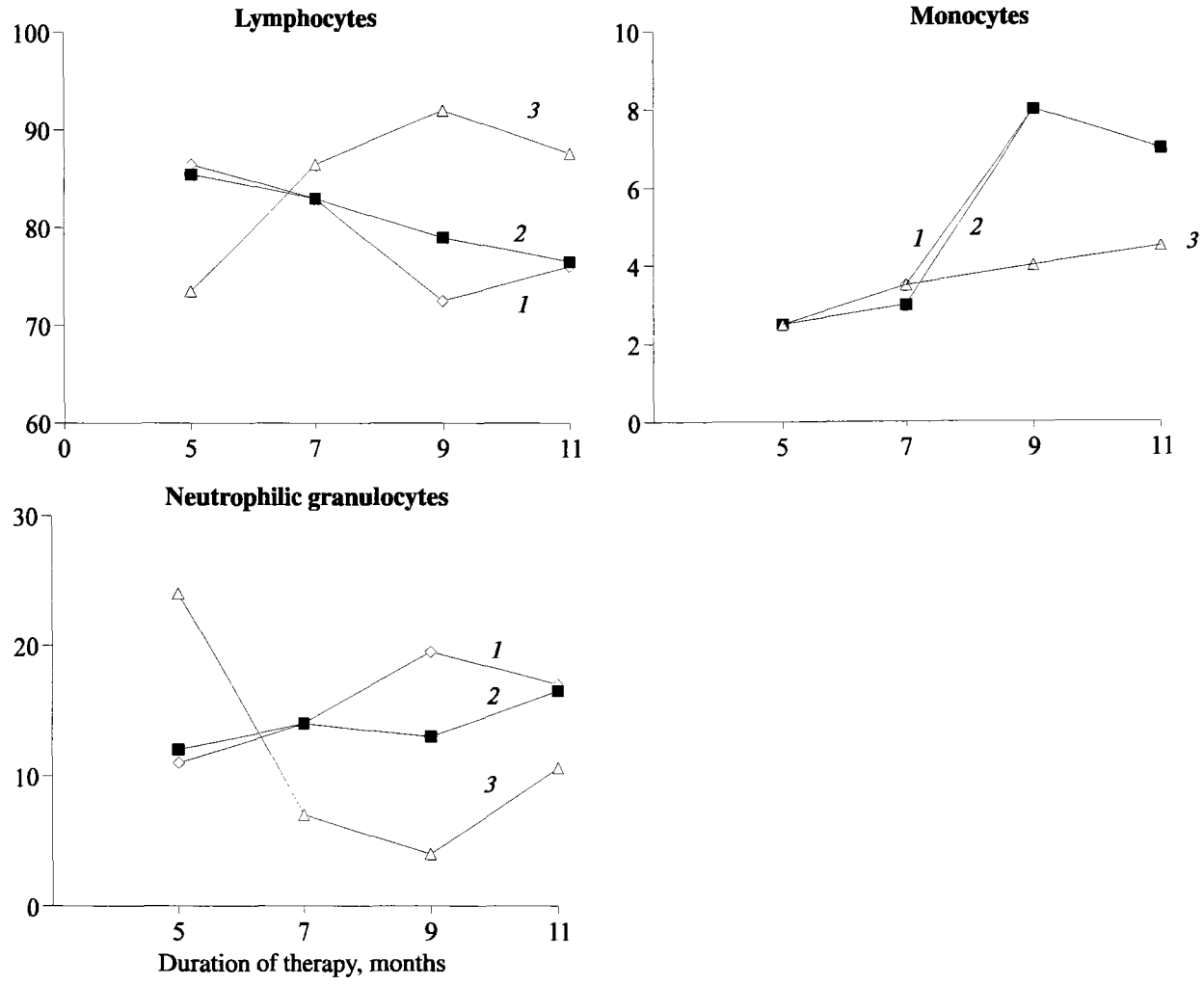

Fig. 3. Contents (%) of lymphocytes, neutrophilic granulocytes, and monocytes in mouse blood.

ted after 7, 9, and 11 months of therapy, but were statistically insignificant. Low number of leukocytes in group 1 mice indicated attenuation of *M. leprae*-induced inflammation.

In groups 1 and 2 mice we also revealed changes in white blood. After 5-month therapy, the percentage of lymphocytes considerably increased, while the content of segmented neutrophils decreased. After 9 and 11 months, the content of NG increased and the percentage of lymphocytes decreased. The content of monocytes considerably increased indicating that these preparations stimulated blood phagocytic activity.

Plasma ALT activity in group 1 mice treated for 9 and 11 months tended to decrease. AST activity in the plasma and ALT and AST activities in the liver remained unchanged to the end of observations.

Hence, HP stimulates MPO in phagocytes (which probably accept the administered enzyme). These changes correlate with phagocytic antimicrobial activity. Long-term therapy with HP produces antiinflammatory effects and does not aggravate functional state of the liver in mice. Changes in red blood cell count provide a reason for detailed studies of the effects of long-term therapy with HP on the state of oxidation-reduction processes in the body. The main purposes of these studies are to prevent side effects of HP therapy and to evaluate the advisability of its use in medical practice.

REFERENCES

1. N. I. Bakhov, L. Z. Aleksandrova, and V. N. Titov, *Lab. Delo*, No. 6, 3-12 (1988).
2. *Laboratory Studies in Clinical Practice* [in Russian], Ed. V. V. Men'shikov, Moscow (1987).
3. A. K. Maslov and A. A. Yushchenko, *Arkh. Pat.*, No. 11, 51-54 (1988).
4. N. V. Beeching and C. J. Ellis, *J. Antimicrob. Chemother.*, **10**, 81-83 (1982).
5. R. C. Graham and M. J. Karnovsy, *J. Histochem. Cytochem.*, **14**, 291-302 (1966).
6. P. Jamet, *Int. J. Lepr.*, **63**, 195-201 (1995).
7. *Laboratory Techniques for Leprosy*, Geneva (1987).
8. C. C. Shepard, *J. Exp. Med.*, **112**, 445-454 (1960).