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Effect of Peroxidase in Complex with Basic Antileprosy Drugs on Liver, Blood, and Functional Activity of Phagocytes in Mice with Experimental Leprosy

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Therapeutic effect of lyophilized horseradish peroxidase in complex with the basic antileprosy drugs diaminodiphenylsulfone and rifampicin was studied in experimental leprosy. Oral therapy with drug complexes was more effective than monotherapy. Treatment with drug combinations activated myeloperoxidase in blood neutrophil, produced an antiinflammatory effect, stimulated cell immunity, and had no toxic effect on mouse liver.

Key Words: *experimental leprosy; peroxidase; diaminodiphenylsulfone; rifampicin; phagocyte activity; complete blood count*

Previously we showed that in mice infected with *Mycobacterium leprae* lyophilized horseradish peroxidase (HP) produced a pronounced antibacterial effect comparable to or even higher than that of the basic antileprosy agent diaminodiphenylsulfone (DDS) [3]. HP given orally with fodder increased myeloperoxidase (MPO) activity in inflammatory infiltrate phagocytes and peripheral blood neutrophils, stimulated cell immunity, and had no toxic effect on mouse liver [4].

The basic antileprosy drugs today are DDS and rifampicin (RFP), and therefore, the aim of this study was to evaluate the antibacterial effects of HP+DDS and HP+RFP combinations on a model of leprosy proposed by C. Shepard [6]. Time course of *M. leprae* levels in mouse paw pads, levels of MPO activity in blood neutrophilic granulocytes, complete blood count, and liver function of mice were studied.

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MATERIALS AND METHODS

CBA mice synchronized for body weight and kept under standard vivarium conditions were infected intraplantarly with a suspension of *M. leprae* (10^4 bacterial cells/mouse) isolated from patients with lepromatous leprosy and passaged twice in laboratory animals. The experiment consisted of two series carried out at different periods. Mice were divided into 3 groups in each series. In series I, the animals were treated with HP (100 U/mg, Merck) and DDS or DDS alone from the moment of infection untill the end of the experiment. In series II, the mice were treated with HP "+" RFP or RFP alone 2 months after infection. Control groups in both series were untreated mice. HP and DDS were given in doses of 150 mg/kg [3] and 100 mg/kg fodder, respectively. RFP was given orally once a week in a dose of 25 mg/kg. The protocols and doses were calculated in accordance with WHO recommendations [5]. The animals (5-6 animals per group) were sacrificed 5, 8, and 11 months after the beginning of experiment and blood, inflammatory infiltrate, and liver were collected.

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The content of *M. leprae* in mouse paws was estimated as described previously [7]. MPO activity in blood neutrophilic granulocyte was evaluated by a semiquantitative method. Blood hemoglobin level, erythrocyte and leukocyte counts, and differential leukocyte count were estimated by universal methods. The state of the liver was assessed by alanine and aspartate aminotransferase activities in the serum and liver homogenate [2]. The results were statistically processed using Statgraphics software and Student's *t* test.

RESULTS

Therapy with HP+DDS combination notably suppressed the growth of *M. leprae* in mouse paws after 5-month therapy. This effect was comparable with that of DDS. After 8 and 11 months the antibacterial effect of the combination was superior to that of monotherapy (Table 1).

The antibacterial effect of HP+RFP combination after 3 months was significantly higher than that of RFP monotherapy (t=3.0; p<0.05). Treatment with this complex for 6 and 9 months almost completely suppressed propagation of *M. leprae* in mouse paws (after 6 months the agent was detected in only one animal, Table 2).

Cytochemical analysis of blood neutrophilic granulocytes from mice receiving drug combinations for a long time showed an essential increase in MPO activity. Therapy with HP+DDS for 8 and 11 months and with HP+RFP for 6 and 9 months produced a more potent antibacterial effect than monotherapy. In addition, 9-month therapy with RFP led to a decrease in MPO activity, which can imply a decrease of their functional potential (Tables 1, 2).

In mice treated with HP+DDS for 5 and 8 months, the content of hemoglobin and erythrocyte count decreased compared to untreated control; the decrease in erythrocyte count after 8 months was statistically significant (t=2.5; p<0.05). Therapy with HP+DDS for 11 months led to a decrease in hemoglobin concentration compared to the control (94.00±16.88 and 158.00± 7.93 g/liter, respectively) and in erythrocyte counts (5.45± 0.33×10^{12}/liter and $9.60\pm0.14\times10^{12}$/liter, respectively).

After 5-month combined therapy with HP+DDS the leukocyte count decreased significantly in comparison with controls and mice receiving DDS monotherapy. A stable decrease in leukocyte count was observed at other periods of the experiment (p<0.05).

The percentage of neutrophilic granulocytes and monocytes increased significantly by month 8 of combined therapy and remained elevated in comparison with the control untill the end of the experiment (p<0.05).

Therapy with HP+RFP for 9 months was associated with a decrease of hemoglobin level and anti-

TABLE 1. Dynamics of *M. leprae* Propagation and MPO Activity in Peripheral Blood Neutrophils in Mice Treated with HP+DDS ($M\pm m$)

Parameter; period of observation, months		Control (no treatment)	DDS	HP+DDS
Number of mycobacteria, 10^5	5	26.84±2.2	7.05±0.87**	8.67±1.06**
	8	333.4±17.6	22.6±2.6*	13.54±3.2**
	11	92.5±12.4	22.94±1.7**	14.48±0.82*
MPO level, arb. units	5	1.70±0.03	1.75±0.05	1.89±0.1
	8	1.67±0.04	1.79±0.04***	2.25±0.15**
	11	1.58±0.02	2.22±0.06**	2.63±0.04**

Note. Here and in Table 2: *p<0.001, **p<0.01, ***p<0.05 *vs.* control.

TABLE 2. Dynamics of *M. leprae* Propagation and MPO Activity in Peripheral Blood Neutrophils in Mice Treated with HP+RFP ($M\pm m$)

Parameter; period of observation, months		Control (no treatment)	RFP	HP+RFP
Number of mycobacteria, 10^5	5	90.5±6.8	9.35±1.29**	4.2±1.12**
	8	1173.1±88.9	0.14±0.14**	0.04±0.04**
	11	6650±858.2	0*	0*
MPO level, arb. units	5	1.66±0.04	2.33±0.09*	2.39±0.05*
	8	1.62±0.12	2.31±0.05*	2.45±0.04*
	11	1.55±0.1	1.94±0.03**	2.48±0.03*

inflammatory effect. No statistically significant fluctuations in the counts of neutrophilic granulocytes and monocytes were detected.

Serum transaminase levels in the blood and liver homogenates of animals treated with HP+DDS and HP+RFP remained stable over the entire experiment.

A notable increase in MPO activity in animals treated with HP+DDS seemed to be due to the fact that both drugs increasing phagocyte MPO activity [4] potentiated the effects of each other. However it was not paralleled by an increase of antibacterial activity (in comparison with DDS) as was observed in HP monotherapy [3].

As immunosuppressants, antibiotics suppress the bactericidal function of blood neutrophilic granulocytes [1]. Complex therapy with HP and RFP promotes an increase in intracellular MPO activity, which activates phagocyte antibacterial activity. Side effect on red blood can be due to exhausting of the antioxidant defense system in cells. Therefore, drug concentrations and terms of treatment should be thoroughly selected.

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