Effects of Horseradish Root on Functional Activity of Phagocytes, Total Blood Cell Count, and State of the Liver in Mice with Experimental Leprosy

A. K. Maslov, S. A. Luzhnova, and O. V. Kalyanina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 8, pp. 181-183, August, 2002
Original article submitted June 13, 2002

Therapy of experimental leprosy with dried and grated horseradish root administered perorally in a dose of 300 mg/kg mixed food and treatment with purified horseradish peroxidase increased myeloperoxidase activity of blood neutrophils, enhanced antimicrobial functions of phagocytes, decreased leukocytosis, normalized total blood cell count, and produced no adverse effects on the functional state of the liver in mice.

Key Words: *experimental leprosy; horseradish root; dynamics of M. leprae reproduction; phagocyte activity; hemogram*

Our previous studies showed that in mice with experimental leprosy (*Mycobacterium leprae*) [7] purified horseradish peroxidase in optimal doses used alone or in combination with antileprotic drugs 4,4'-diaminodiphenyl sulfone (DDS) and rifampicin markedly inhibits the growth of *M. leprae* and increases phagocyte myeloperoxidase (MP) activity. Horseradish peroxidase possesses antiinflammatory activity, stimulates cell immunity, and has no effect on the functional state of the liver in mice [3-5].

Peroxidases are abundant in animals and plants. Horseradish root (HR) is widely used at the source of peroxidases and provides the highest yield of this enzyme.

Here we studied the possibility of using HR for the therapy of experimental leprosy.

MATERIALS AND METHODS

Experiments were performed on CBA mice of the same weight kept under standard conditions. The mice were infected by intraplantar injection of *M. leprae* suspension (10^4 bacterial bodies per mouse) isolated from patients with lepromatous leprosy and passed 2 times in experimental animals. It should be emphasized that the experiments were performed at various seasons and *M. leprae* were isolated from different patients (various bacterial strains), therefore the intensity of bacterial reproduction varied. The mice of each group were infected with the same strain of *M. leprae*. After treatment the mice fed a diet containing 100, 300, and 500 mg dried and grated HR per 1 kg mixed food. The roots were dried at room temperature, ground in an electrical coffee grinder, and stored at 4°C.

Control animals were infected with *M. leprae* and received DDS in a dose of 100 mg/kg food. These preparations were tested by conventional methods recommended by the World Health Organization [6]. The mice (5-6 per group) were decapitated 5, 8, and 11 months after the start of experiments to obtain the blood, inflammatory infiltrate, and liver.

The number of *M. leprae* in mouse paws was estimated as described elsewhere [8]. MP activity in blood neutrophilic granulocytes, the content of hemoglobin, number of erythrocytes and leukocytes, and differential leukocyte count were estimated routinely. Functional activity of the liver was estimated by activities of alanine and aspartate transaminases (ALT and AST, respectively) in plasma and liver homogenates [2].

Institute for Leprosy, Russian Ministry of Health, Astrakhan. ***Address for correspondence:*** niil@astmail.astranet.ru. Maslov A. K.

0007-4888/02/1342-0156$27.00

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

RESULTS

HR in doses of 100, 300, and 500 mg/kg food effectively inhibited the growth of *M. leprae* in mice, which was especially pronounced 5 months after the start of therapy (by 9-20 times). The therapy with HR in a dose of 100 mg/kg food for 5, 8, and 11 months produced an antimicrobial effect, which was similar or less pronounced than the effect DDS. HR in a dose of 300 mg/kg food most significantly inhibited the growth of *M. leprae* (compared to DDS and untreated mice). After 5-month therapy with HR and DDS the number of *M. leprae* in mouse paws was 14.6 and 6.14 times lower than in untreated animals, respectively. After 8 and 11 months of therapy, the antimicrobial effect of HR surpassed that of DDS. HR in a dose of 500 mg/kg food produced a less pronounced therapeutic effect than in a dose of 300 mg/kg (Table 1).

Cytochemical examination of neutrophilic granulocytes showed that HR and DDS markedly increased intracellular MP activity (compared to the control). MP activity in neutrophilic granulocytes increased with increasing the dose of HR (compared to treatment with DDS) and after 11 months enzyme activity in mice receiving HR in a dose of 500 mg/kg food significantly surpassed that in animals treated with DDS ($p<0.02$). In control mice MP activity in neutrophilic granulocytes decreased during this period (Table 2). In mice receiving HR in a dose of 500 mg/kg food, no correlation was found between activation of MP and the increase in antibacterial activity of phagocytes.

HR and purified horseradish peroxidase possessed antiinflammatory activity, stimulated cell immunity, and produced no toxic effect on the liver. After 5 months, HR in the maximum effective dose (300 mg/kg food) significantly decreased leukocyte count compared to that in controls and DDS-receiving animals. The decrease in leukocyte count was also observed in other periods of observations ($p<0.01$). The number of neu-

TABLE 1. Reproduction of *M. leprae* in Mouse Paws (10^6 Mycobacteria, $M\pm m$)

HR dose, mg/kg; time after inoculation, months		Control	Therapy	
			DDS	HR
100	5	2.05±0.22	0.16±0.05***	0.10±0.03***
	8	6.85±0.96	2.67±0.98***	3.79±0.90
	11	7.43±1.54	2.00±0.68***	2.40±0.78***
300	5	39.58±5.90	6.44±1.07**	2.71±1.54*
	8	98.32±6.09	45.27±5.70*	13.10±5.13*
	11	113.7±15.9	51.83±9.12**	20.5±5.4*
500	5	21.39±4.20	3.98±0.90**	2.35±0.40**
	8	62.3±11.1	16.5±2.8**	10.18±1.30*
	11	117.2±11.8	27.53±3.70*	19.27±5.80*

Note. Here and in Table 2: *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control.

TABLE 2. MPO Activity in Peripheral Blood Neutrophils in Mice (rel. units, $M\pm m$)

HR dose, mg/kg; time after inoculation, months		Control	Therapy	
			DDS	HR
100	5	1.73±0.05	1.98±0.06**	2.03±0.02*
	8	1.65±0.03	2.14±0.05*	2.13±0.02*
	11	1.58±0.05	2.25±0.05*	2.37±0.06*
300	5	1.78±0.06	1.96±0.04***	2.04±0.04**
	8	1.68±0.02	2.11±0.08**	2.25±0.02*
	11	1.59±0.04	2.23±0.08*	2.35±0.06*
500	5	1.75±0.12	2.05±0.04	2.26±0.09**
	8	1.68±0.03	2.22±0.07*	2.43±0.18**
	11	1.56±0.01	2.25±0.06*	2.59±0.1*

trophilic granulocytes and monocytes significantly increased by the 8th month of treatment with HR and surpassed the control ($p<0.05$). As differentiated from horseradish peroxidase and DDS, long-term therapy with HR did not decrease erythrocyte count and hemoglobin amount in the blood.

ALT and AST activities in mice treated with HR in various doses did not differ from those in control and intact animals. Thus, long-term therapy with the test preparation produced no toxic effects on mouse liver.

Our results indicate that dried and grated HR administered perorally in a dose of 300 mg/kg food produces a potent antimicrobial effect in mice with experimental leprosy (similarly to pure horseradish peroxidase [3,4]). This is probably associated with high content of peroxidase activating the MP system in phagocytes, the main bactericidal system. When activity of intracellular MP increases, highly active antibacterial products can produce a cytopathic effect on cells membranes and cause their destruction or dysfunction [1]. These changes can explain not only the decrease in antimicrobial phagocytic activity produced by HR in high dose, but also the development of anemia after treatment with purified horseradish peroxidase and DDS [4]. Normalization of erythrocyte count after administration of HR to animals with experimental leprosy was probably related to the presence of ascorbic acid in high concentrations (110-200 mg/100 g), which possesses pronounced reparative activity and promotes stabilization of cell membranes. Moreover, ascorbic acid reduces utilization of vitamin E.

These data indicate that HR can be used as a food additive in the therapy of patients with leprosy (instead of DDS).

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*, Ed. V. V. Men'shikov [in Russian], Moscow (1987).
3. A. K. Maslov and O. V. Kalyanina, *Byull. Eksp. Biol. Med.*, **129**, No. 5, 571-573 (2000).
4. A. K. Maslov and S. A. Luzhnova, *Ibid.*, **130**, No. 7, 83-87 (2000).
5. A. K. Maslov, S. A. Luzhnova, and O. V. Kalyanina, *Ibid.*, **132**, No. 11, 551-553 (2001).
6. *Laboratory Techniques for Leprosy*, Geneva (1987).
7. C. C. Shepard, *J. Exp. Med.*, **112**, 445-454 (1960).
8. C. C. Shepard and D. H. McRae, *Int. J. Lepr.*, **36**, 78-82 (1968).