# Effect of Peroxidase on the Development of Experimental Leprosy in Mice after Intraplantar Infection

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Lyophilized horseradish peroxidase (activity 100 U/mg) administered *per os* in a dose of 100-200 mg/kg fodder enhanced bactericidal activity of phagocytes in mice experimentally infected with *Mycobacterium leprae*, which manifested in suppression of *M. leprae* growth in comparison with untreated controls.

**Key Words:** *peroxidase; experimental leprosy; time course of M. leprae multiplication*

Improvement of diagnosis and development of therapeutic methods aimed at the correction of impaired immunoreactivity necessitate detailed studies of pathogenesis of immunodeficiencies.

Acid hydrolases in phagocyte phagolysosomes destroy only the bacteria killed by the myeloperoxidase system, nonenzymatic cationic proteins, lysozyme, and lactoferrin [8]. Acquired or hereditary myeloperoxidase (MPO) deficiency in the mononuclear phagocyte system is responsible for chronic infectious and granulomatous diseases [2,3,10].

In armadillos with experimental leprosy, *Mycobacterium leprae* were not found in granuloma macrophages with high MPO activity, while macrophages with low MPO activity contained numerous intact *M. leprae* [13].

Electron microscopy of skin granulomas from patients with lepromatous leprosy showed accumulations of morphologically intact *M. leprae* in the cytoplasm of macrophages with low MPO activity and active lysis of bacteria in macrophages with high MPO activity [5].

Long-term (more than 16 years) analysis of the rate and stability of regression of lepromatous process during therapy showed that high MPO activity in granuloma macrophages correlated with rapid elimination of the agent from the organism and high efficiency of therapy, while low activity of macrophagal MPO correlated with slow regression of the disease during therapy and risk of leprosy relapse [6].

Experimental inhibition of MPO activity in phagocytes (mouse peritoneal macrophages) led to long-term persistence of pathogenic microorganisms (*M. leprae* and *M. tuberculosis*) in their cytoplasm [4], while inhibition of phagocyte MPO activity in mice with experimental leprosy resulted in a more rapid propagation of *M. leprae* in comparison with common infection and generalization of the process (involvement of lung and spleen tissue) [7].

These data indicate that MPO activity is a reliable criterion for evaluating the phagocytic capacity.

This prompted us to use lyophilized horseradish peroxidase (HRP) for enzymatic therapy of experimental leprosy in mice [14].

## MATERIALS AND METHODS

CBA mice ($n$=450) initially weighing 25-30 g were used. A suspension of *M. leprae* ($10^4$ bacterial cells/mouse) isolated from patients with lepromatous leprosy (1-2 passages in animals) was injected into the right hind paw pad. Since the bacteria were isolated from different patients, their multiplication rates varied. The same strain was used in each group of mice.

After infection, the mice were fed a mixture containing 50, 100, 150, 200, and 250 mg lyophilized HRP (Merck, 100 U/ml) per kg fodder.

The mice infected with *M. leprae* treated with the basic antileprosy drug diaminodiphenylsulfone (DDS)

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in a dose of 100 mg/kg fodder. Untreated mice were controls. Fodder-drug mixture was given to mice during the entire experiment. The drug was tested in accordance with WHO recommendations [12].

The animals were decapitated 5, 8, and 11 months after infection (5-6 mice per group). *M. leprae* were counted in mouse paws as described previously [15]. The results were processed using Student's *t* test.

## RESULTS

Horseradish peroxidase effectively inhibited bacterial growth. After 5-month treatment with HRP in doses of 150 and 200 mg/kg fodder, the number of *M. leprae* in mouse paws was 4-5-fold lower, while after DDS treatment this parameter decreased only 2-3-fold (Table 1). After 8 months the antibacterial effect of HRP became even more pronounced: the number of *M. leprae* in the paws was 2-13 times lower than in the untreated controls. The same was observed in mice treated with HRP in a dose of 100 mg/kg fodder (Table 1). In animals treated with DDS, the number of *M. leprae* was 4-6-fold higher than in mice treated with HRP. After 11 months of treatment with HRP the trend to suppressing the growth of *M. leprae* remained at the same level. HRP doses 50 and 250 mg/kg fodder were less effective (Table 1).

Hence, the most stable inhibition of *M. leprae* growth in mouse paw pads were observed at HRP doses of 100-200 mg/kg fodder given orally from the moment of infection until the end of the experiment.

The bactericidal properties of phagocytic cells largely depend on production of active oxygen. MPO deficiency is associated with decreased release of active oxygen by phagocytes. Experiments showed that even trace ($3\times10^{-4}$ M) concentration of HRP increased phagocyte chemiluminescence, which was associated with their activation during disintegration of the phagocytosed material [11].

MPO activity is present in monocytes and their precursors and disappears during monocyte differentiation into macrophages. On the other hand, MPO is present in neutrophilic granulocytes in all mammals and humans [10]. Experiments demonstrated that macrophages phagocytizing neutrophilic granulocytes degradation products rich in MPO acquired new properties and their functional activity increased. Therefore, when evaluating cell defense reactions, one should study the granulocytic and macrophagal systems, which are in a state of permanent interactions. After a short active period granulocytes in tissues are destroyed and phagocytosed by macrophages, thus modifying their properties. The stimulating effect on macrophages is due to consumption of MPO rendering antibacterial activity [9].

The therapeutic effect of MPO in experimental leprosy infection suggests that increased functional activity of macrophages is explained by penetration of

**TABLE 1.** Time Course of *M. leprae* Multiplication in Mouse Paws ($10^8$ Mycobacteria, *M±m*)

| HRP dose, mg/kg | Term after infection, months | Untreated control | Therapy | |
|---|---|---|---|---|
| | | | DDS | RHP |
| 50 | 5 | 1.005±2.600 | 0.305±0.156 | 0.728±0.161 |
| | 8 | 7.85±2.93 | 2.730±0.795 | 3.480±0.715 |
| | 11 | 5.512±1.110 | 2.480±0.319* | 2.320±0.275* |
| 100 | 5 | 4.2±2.0 | 3.0±1.5 | 2.8±1.1 |
| | 8 | 285±109 | 96.0±18.4* | 19.9±9.6* |
| | 11 | 186.2±54.0 | 53.4±14.1* | 9.7±3.5* |
| 150 | 5 | 22.50±5.27 | 9.28±2.47 | 5.21±1.71** |
| | 8 | 140.7±32.9 | 41.92±10.64* | 10.75±3.09*** |
| | 11 | 116.2±32.8 | 31.54±10.80* | 7.51±2.02** |
| 200 | 5 | 17.51±4.70 | 6.30±1.04 | 3.25±1.57** |
| | 8 | 88.2±12.8 | 36.50±9.13 | 7.02±2.68* |
| | 11 | 61.25±7.34 | 15.54±5.65* | 3.50±1.53** |
| 250 | 5 | 20.30±5.18 | 4.08±0.30* | 3.50±0.71* |
| | 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 |

**Note.** *$p<0.01$, **$p<0.02$, ***$p<0.05$ *vs.* control.

peroxidase into these cells and assembly of Klebanov's antibacterial system (peroxidase+$H_2O_2$+I).

Absorption of low molecular weight enzymes, such as HRP, in the gastrointestinal tract has been reported [1].

The results prompt purposeful search for clinical application of peroxidase.

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