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Study of Antileprosy Activities of Some Dialkyldithiocarbamate Derivatives

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Effects of some dialkyldithiocarbamate derivatives on multiplication of *M. leprae* were studied in infected mice. Compounds significantly suppressing *M. leprae* proliferation were selected. By antibacterial activity one of these compounds was superior to dapsone, the main antileprosy drug.

Key Words: *M. leprae; dialkyldithiocarabamate derivatives; dapsone; antileprosy activity*

The Leprosy Elimination Campaigns, unfold by the WHO in 1991 and aimed at reducing the prevalence of this disease in countries with endemic lepra to one patient per 10,000 population by the year 2000, is based on provision of effective modern drugs for all patients. In 1999 it became clear that this goal could not be attained in all countries of the world because of the absence of appropriate infrastructure, insufficient available data on the number of patients, political instability, migration processes, *etc.* For this reason the WHO and the International Alliance of Governmental and Social Organization, created under the WHO aegis, delayed the term of this goal achievement till 2005 [1]. However, the problem remains unsolved up to the present time: 500,000-800,000 new cases of leprosy are annually registered in the world; new, heretofore unknown, foci of infection are detected and the reports about relapses in patients treated by the WHO protocols within the LEC program are published more and more often [2-4]. The therapy efficiency is the factor, determining the fate of not only the patient and his or her relatives, but also the epidemiological prospects of the place of his/her residence.

Since the beginning of 1980s the main method for the treatment of leprosy patients all over the world is combined therapy by the WHO protocol: simultaneous prescription of two or three antileprosy drugs, depending on the disease type (dapsone, rifampicin, and clofazimine for patients with multibacillary leprosy and dapsone and rifampicin for paucibacillary leprosy). Dapsone, a sulfone drug characterized by bacteriostatic and antiinflammatory activity, transforms leprosy from an incurable disease into a curable one and remains the major antileprosy drug. Rifampicin is a drug with high bactericidal activity. Clofazimine, in addition to bactericidal effect towards *M. leprae*, is characterized by a pronounced antiinflammatory effect, and is therefore preferable in leprous neuritis. Minocyclin, ofloxacin, clarithromycin, suggested as alternatives to the main drugs, did not justify the hopes.

Even with effective drugs, the periods of therapy of patients with leprosy remain long. At least two years are needed to attain clinical and bacterioscopic regression in multibacillary leprosy and

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at least 6 months in paucibacillary forms, and the patients with multibacillary forms have to receive antirelapse therapy in fact for the whole life. The available antileprosy drugs do not solve the problem of leprous reactions, which can result in involvement of the peripheral nervous system, often leading to severe disability. They are ineffective towards persistent mycobacteria. For these reasons the search for drugs suppressing multiplication of *M. leprae* and creation of new antileprosy drugs remain the pressing problems of leprology.

We studied the capacity of some dialkyldithiocarbamate derivatives (synthesized at Institute of Antibiotics), *in vitro* inhibiting multiplication of *M. tuberculosis*, to suppress multiplication of *M. leprae*.

MATERIALS AND METHODS

Antileprosy activity of three dialkyldithiocarbamate derivatives was studied in experiments on CBA mice infected with *M. leprae* in a dose of 5×10^3 in the paw pads by the method described previously [5,6] and recommended by the WHO for screening of the substances inhibiting multiplication of the leprosy agent. The quantity of mycobacteria in 1.0 ml suspension used for infection and obtained from animal soft tissues was evaluated as described previously [7].

Experiment was carried out on 135 mice (18-22 g) infected in the right hind paw by *M. leprae* strain isolated from an untreated patient with lepromatous leprosy and passaged in mice at Institute of Leprosy Research. All studied substances and dapsone (reference drug) were administered through a tube in doses of 10 and 30 mg/kg 5 times a week from the day of infection. Animals of groups 1 and 2 received 2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine, groups 3 and 4 received 2-cyano-4,6-dinitrophenyl-N,N-dimethylcarbamodithionate, groups 5 and 6 received 3,5-dinitro-2-tetramethylenedithiocarbamoylpyridine, and groups 7 and 8 received dapsone. Group 9 animals receiving no treatment served as controls. Each group consisted of 15 mice. The duration of the experiment was 7.5 months, after which the animals were sacrificed. After decapitation soft tissues of the right hind paws were resected, homogenized, and suspension for counting *M. leprae* was prepared. The capacity of the compounds to suppress multiplication of *M. leprae* was evaluated by the difference between the content of bacteria in the paw tissues from mice treated with these compounds and controls, while the efficiency of these compounds was evaluated by the difference in the parameters in comparison with animals treated with dapsone. The results were processed using Student's test.

RESULTS

The mean content of *M. leprae* 7.5 months after infection in controls was $(3.91\pm0.33)\times10^5$. This value was significantly lower in both groups of mice treated with dapsone (Table 1).

2-Diethyldithiocarbamoyl-3-cyano-5-nitropyridine exhibited the highest activity in suppression of *M. leprae* multiplication in comparison with other studied compounds. The mean number of *M. leprae* in mice treated with this substance was significantly lower than in the control. Antileprosy activity of 2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine in a dose of 10 mg/kg was somewhat lower than that of dapsone in the same dose. Antileprosy activity of this compound in a dose of 30 mg/kg was significantly higher than that of dapsone ($p<0.01$). The efficiency of 2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine in suppression of mycobacteria multiplication was markedly dose-dependent: a 3-fold increase of the dose led to a 10-fold decrease in the mean number of *M. leprae* ($p<0.01$).

TABLE 1. Antileprosy Activity of the Studied Compounds

Compound	Dose, mg/kg	Number of *M. leprae* in paw pads (10^5)
Control		3.91±0.33
2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine	10	0.56±0.11*
	30	0.06±0.01*
2-cyano-4,6-dinitrophenyl-N,N-dimethylcarbamodithionate	10	0.83±0.15*
	30	0.77±0.10*
3,5-dinitro-2-tetramethylenedithiocarbamoylpyridine	10	2.13±0.28*
	30	1.83±0.20*
Dapsone	10	0.27±0.07*
	30	0.22±0.04*

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

The mean number of *M. leprae* in mice treated with 2-cyano-4,6-dinitrophenyl-N,N-dimethylcarbamodithionate was significantly lower in the control group, but higher than in animals treated with dapsone and 2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine. A 3-fold increase of the dose did not lead to a decrease in the number of *M. leprae*.

The mean number of *M. leprae* in mice treated with 3,5-dinitro-2-tetramethylenedithiocarbamoylpyridine was significantly lower than in the control, but higher than in animals treated with 2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine, 2-cyano-4,6-dinitrophenyl-N,N-dimethylcarbamodithionate, and dapsone.

Hence, all studied dialkyldithiocarbamate derivatives exhibited antibacterial activity towards *M. leprae*. 2-Diethyldithiocarbamoyl-3-cyano-5-nitropyridine and 2-cyano-4,6-dinitrophenyl-N,N-dimethylcarbamodithionate most effectively inhibited multiplication of *M. leprae*, the former of these compounds in a dose of 30 mg/kg exhibited high antileprosy activity far surpassing that of dapsone in the same dose. These results suggest good prospects of further studies of the antileprosy activity of these substances and other dithiocarbamate derivatives. 2-Diethyldithiocarbamoyl-3-cyano-5-nitropyridine attracts special interest, because it belongs to a different chemical group than dapsone, which suggests different mechanism of the antibacterial effect. This compound as a perspective drug for combined use with dapsone and its alternative use in cases with dapsone resistance.

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