Antileprosy Activity and Preliminary Toxicological Characteristics of Some Dialkyldithiocarbamate Derivatives

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 4, pp. 424-426, April, 2008
Original article submitted November 29, 2007.

Antileprosy activity of dialkyldithiocarbamate derivatives was studied in experiments on mice infected with *M. leprae* into paw pads. We found that 2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine is the most promising antileprosy agent; it effectively suppresses multiplication of *M. leprae* and is well tolerated under conditions of chronic animal experiment.

Key Words: *M. leprae, dialkyldithiocarbamate; antileprosy activity; preliminary toxicological characteristics*

The number of leprosy patients with not only mono-, but also multiresistsznce to antileprosy drugs (dapsone, rifampicin, and lamprene) is increasing during recent years, which determines increased incidence of relapses and activation of existing and appearance of new nidi of the disease. Therefore, the search and creation of new drugs close to the known preparation by their efficiency, but belonging to other chemical groups is an urgent problem of leprology [1,4]. Previous experiments showed that dithiocarbamate (DC) derivatives suppress proliferation of *M. leprae* in mouse paw pads [2,3]. Moreover, 2-diethyldithiocarbamoyl-3-cyano-5-nitropyridine (DC-1) by its antileprosy activity was close to dapsone, the major component of combined therapy schemes in leprosy. For the search of promising candidates for antileprosy drugs we studied antibacterial activity against *M. leprae* and tolerability of 6 new DC (of them 3 preparations were 3-cyano-5-nitropyridine derivatives similarly to DC-1; all preparations were synthesized in Research Center for Antibiotics).

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MATERIALS AND METHODS

The study was performed on 538 CBA male mice weighing 20-22 g infected with *M. leprae* in a dose of 5×10^3 into paw pads [5]. The mice were infected with a *M. leprae* strain isolated from a patient with lepromatous leprosy. The test substances (2-heptamethylene-dithiocarbamoyl-3-cyano-5-nitropyridine — DC-2; 2-hexamethylene-dithiocarbamoyl-3-cyano-5-nitropyridine — DC-3; 2-pyrrolidine-dithiocarbamoyl-3-cyano-5-nitropyridine — DC-4; 4-pyrrolidine-dithiocarbamoyl-5-nitro-6-dimethylaminopyrimidine — DC-5; 4-pyrrolidine-dithiocarbamoyl-5-nitro-6-methoxypyrimidine — DC-6; 2-pyrrolidine-dithiocarbamoyl-3,5-dinitrobenzamide — DC-7) and reference preparations dapsone and DC-1 were administered through a gastric tube 5 times a week starting from the day of infection. Infected untreated animals served as the control.

A suspension was prepared from soft tissues of mouse paw pads. Suppression of *M. leprae* multiplication was evaluated by the number of mycobacteria in paw tissues of treated and control mice, the efficiency of the test preparations was assessed by the differences in this parameter from the groups

0007-4888/08/1454-0446

TABLE 1. Animal Mortality (%) after Treatment with DC Derivatives in Initial Doses

Compound	Dose, mg/kg	
	10	30
DC-1*	0	—
DC-2	47	63
DC-3	47	47
DC-4	36	42
DC-5	26	26
DC-6	0	15
DC-7	36	63

Note. *DC-1 was administered in a dose of 20 mg/kg

receiving dapsone or DC-1. For preliminary toxicological evaluation of the test preparations, biochemical parameters of blood serum (ALT, AST, bilirubin, creatinine, urea) were determined on a Mitsubishi Super Z-818 analyzer (reagents were from ECO-MED-POLL company).

The data were processed statistically using Student *t* test.

RESULTS

In series I, the test compounds were administered in doses of 10-30 mg/kg. Each group consisted of 19 animals. Three weeks after the start of the experiment, animal mortality was observed in the group treated with DC-2-DC-7. The treatment was discontinued on day 26 after the start of treatment, but the animals still died. By the middle of week 5, animal mortality in these groups varied from 15% (DC-6 in a dose of 30 mg/kg) to 63% (DC-2 in a dose of 30 mg/kg). None animals died in the control group and group treated with dapsone and DC-1 (Fig. 1).

In series II, the doses were reduced 5-fold (*i.e.* to 2-6 mg/kg). The experimental groups consisted of 7-18 animals. After 7 months the animals were decapitated. Signs of the toxic effect of the test preparations were not observed after reduction of the doses (Table 2).

TABLE 2. Antileptosy Activity of DC derivatives (Number of *M. leprae*, $\times 10^7$; *M*±*m*)

Compound	Dose, mg/kg	
	2	6
Control	2.71±0.49	—
Dapsone	0.025±0.005	—
DC-1*	0.042±0.014**	—
DC-2	6.64±0.32**	0.28±0.06**
DC-3	0.56±0.17**	0.36±0.07**
DC-4	4.43±0.65**	2.14±0.30
DC-5	0.07±0.02**	0.029±0.006**
DC-6	0.66±0.12**	0.020±0.006**
DC-7	1.27±0.16**	0.56±0.16*

Note. *DC-1 was administered in a dose of 4 mg/kg, **$p<0.05$ compared to the control.

TABLE 3. Biochemical Parameters of Blood Serum in Animals Receiving DC Derivatives (*M*±*m*)

Compound	Dose, mg/kg	AST, U/liter	ALT, U/liter	Bilirubin, μmol/liter	Urea, μmol/liter	Creatinine, μmol/liter
Control		210.80±7.94	48.82±1.65	6.35±0.34	14.33±0.41	6.45±0.27
Dapsone	2	219.73±5.89	51.93±1.25	6.68±0.30	14.57±0.59	6.18±0.42
DC-1	4	214.60±3.94	48.72±1.79	5.56±0.27	13.64±0.33	5.69±0.31
DC-2	2	240.87±4.07*	53.47±0.60*	7.29±0.25*	16.72±0.29***	7.59±0.28*
	6	241.18±3.21*	54.42±0.68*	7.69±0.30**	17.36±0.33***	7.62±0.35*
DC-3	2	228.00±2.95	52.91±1.27	7.21±0.28	15.72±0.57	6.90±0.25
	6	233.74±2.26*	54.27±0.50*	7.32±0.33*	16.42±0.65*	6.98±0.59
DC-4	2	219.03±8.30	52.03±1.08	6.55±0.33	14.75±0.58	6.42±0.34
	6	232.16±2.49*	53.10±0.94	7.00±0.39	14.91±0.74	6.68±0.30
DC-5	2	229.02±4.36	48.11±2.64	6.39±0.38	13.76±0.45	5.95±0.22
	6	228.13±4.19	49.98±1.51	6.57±0.33	14.84±0.36	6.33±0.27
DC-6	2	213.23±7.92	48.72±1.21	5.78±0.29	13.98±0.44	5.91±0.31
	6	217.2±9.31	49.98±0.74	5.99±0.35	14.38±0.30	6.09±0.19
DC-7	2	212.78±8.69	50.13±1.63	6.30±0.26	14.55±0.26	6.26±0.35
	6	226.25±2.96	49.98±0.81	6.59±0.41	15.32±0.74	6.09±0.14

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control.

Modification of the structure of 3-cyano-5-nitropyridine (addition of chemical groups not present in the parent compound) did not potentiate antileprosy activity of the test preparations. Moreover, the number of *M. leprae* in paw pads of mice receiving DC-2 and DC-4 in a dose of 2 mg/kg surpassed the control value. DC-3 moderately suppressed multiplication of mycobacteria, but its antileprosy activity was lower than that of dapsone and DC-1.

The maximum suppression of *M. leprae* multiplication was observed after treatment with DC-5 (2 and 6 mg/kg) and DC-6 (6 mg/kg). Their antileprosy activity was comparable to that of dapsone (2 mg/kg) and DC-1 (4 mg/kg).

Biochemical parameters of the serum in animals receiving dapsone, DC-1, DC-5, DC-6, and DC-7 were close to the control values (Table 3). After treatment with DC-4, a tendency to transaminase elevation was observed. After treatment with DC-4 in a dose of 6 mg/kg, AST activity was significantly elevated. Pronounced shifts in biochemical parameters were observed in animals receiving 6 mg/kg DC-3 and DC-2 in both doses.

Thus, DC-1, DC-5, and DC-6 in doses of 2 and 6 mg/kg were most active against *M. leprae* and had no toxic effects on the studied blood parameters. However, animal death in groups treated with DC-5 at the initial stages of the experiment suggests that the toxic (10 mg/kg) and effective (2-6 mg/kg) doses of this compound are close. Administration of DC-6 in a dose of 30 mg/kg also led to animal death (15%), while the dose of 10 mg/kg was well tolerated. The maximum antileprosy effect was attained after administration of 6 mg/kg DC-6. The tested DC derivatives did not exceed DC-1 by antileprosy activity. Therefore, DC-1 is still the most promising antileprosy agent, because it most potently suppressed multiplication of *M. leprae* and was well tolerated under conditions of long-term treatment.

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