Effect of Liposomal L-Dopa on the Parkinson's Syndrome in Mice

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The effects of free L-Dopa and L-Dopa enclosed in small monolamellar liposomes consisting of egg phosphatidylcholine and cholesterol (molar ratio 7:3) on the severity of Parkinson's syndrome are studied. The syndrome is modeled in C57Bl/6 mice by repeated injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). A single injection of liposomes with L-Dopa on day 7 after the start of MPTP administration has no effect on this syndrome. Repeated injections (for 14 days) of L-Dopa solution (50 mg/kg) into MPTP-treated mice with oligokinesia and muscle rigidity increase their locomotor activity and the number of upright postures 7-fold and decrease muscle rigidity for 1 h after the last injection. The inhibitory effect of a 10-fold lower dose of liposomes with L-Dopa on oligokinesia and muscle rigidity is 2- to 3-fold stronger and 5-7 h longer compared with that of free L-Dopa.

Key Words: *Parkinson's syndrome; MPTP; liposomal L-Dopa; mice*

Parkinson's syndrome (PS) is caused by sharp decrease in the dopamine concentration in the striatum resulting from the damage to dopaminergic neurons in the substantia nigra [1,7]. Since dopamine does not cross the blood-brain barrier, its precursor L-Dopa has been used in medical practice for the treatment of PS [1,2,5,12]. However, with progression of the disease, treatment with L-Dopa induces a number of adverse reactions (for example, dyspepsia and cardiovascular disturbances) associated with its peripheral decarboxylation [5]. Administration of L-Dopa in high doses and the development of hypersensitivity by postsynaptic receptors to it [10] lead to psychotic and neurological complications such as dyskinesia, visual hallucinations, dementia, choreiform syndrome, etc. [5,8,13].

Although considerable progress has been achieved in the treatment of parkinsonism with dopaminergic drugs [1,2,13], it is important to find more effective and prolonged drugs without adverse effects. It was demonstrated that encapsulation into liposomes protects drugs against degradation, which increases their therapeutic effectiveness at a lower dose [6,9,11,15]. Therefore, liposomes may provide more effective transport of L-Dopa into the brain, increase its concentration in the striatonigral system, and reduce its adverse effects. In the present study we compared the effect of L-Dopa encapsulated in small monolamellar liposomes (SML) with that of free L-Dopa on the development of PS in mice.

MATERIALS AND METHODS

C57Bl/6 mice weighing 23-25 g were used. Parkinson's syndrome was induced by administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, intraperitoneal injections in a dose of 20 mg/kg twice daily at a 12-h interval for 21 days). Liposomes were prepared by sonicating multilamellar liposomes consisting of phosphatidylcholine

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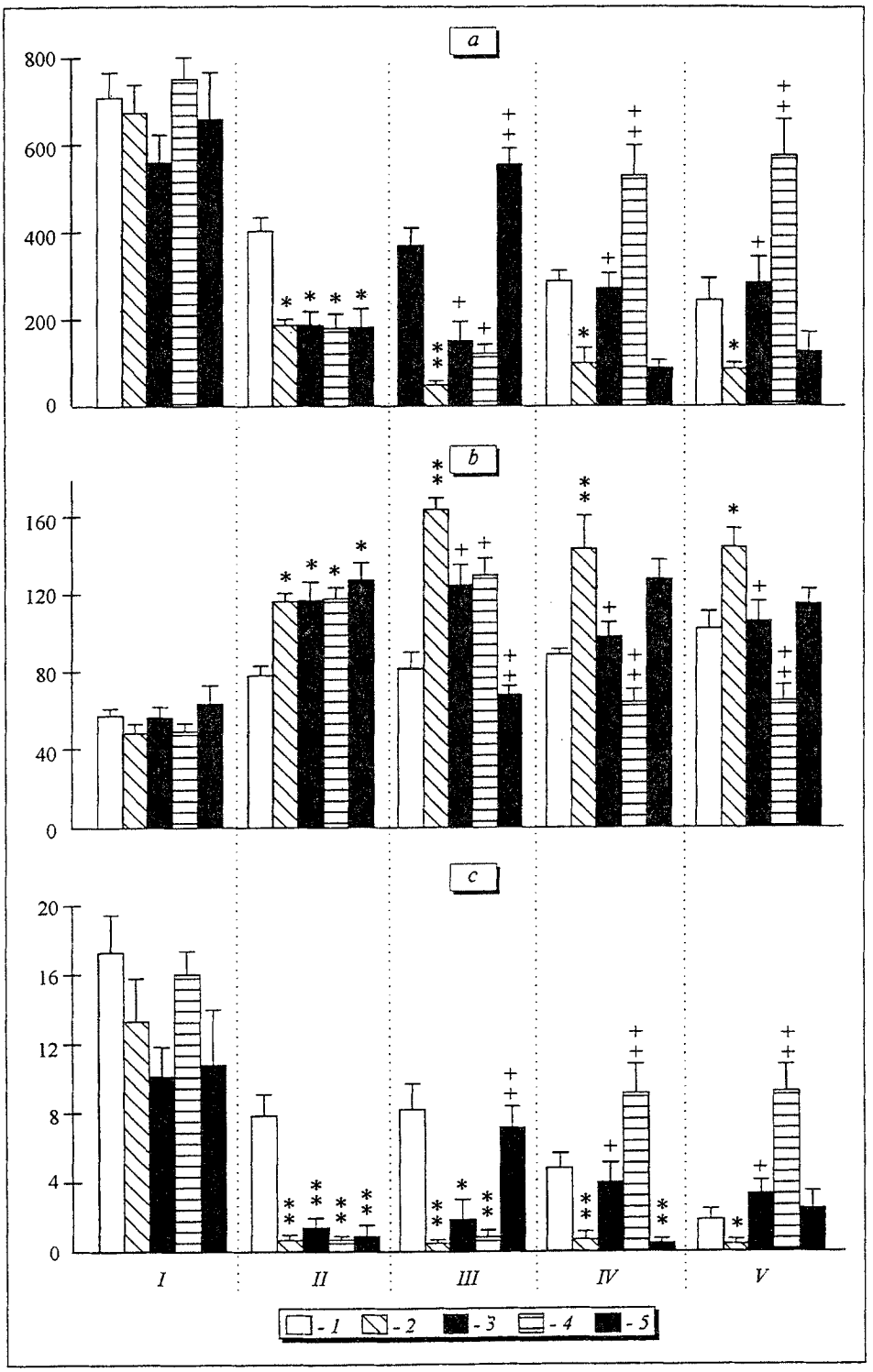

Fig. 1. Effects of free L-Dopa and L-Dopa-containing small monolamellar liposomes (SML) on the locomotor activity (*a*), duration of immobility (*b*), and vertical movements (number of upright postures, *c*) in mice treated with MPTP. Ordinate: *a*) distance covered by mice for 3 min, cm; *b*) duration of immobility for 3 min, sec; *c*) number of upright postures within a 3-min period. Observation times: *I*) before MPTP treatment; *II*) on day 7 of MPTP treatment; *III*, *IV*, and *V*) at 1 h, 3 h, and 5 h after injection of SML without L-Dopa, L-Dopa-containing SML, and free L-Dopa, respectively, on day 21 of MPTP administration. *1*) mice given 0.9% NaCl; *2*) mice given MPTP and 0.9% NaCl; *3*) mice given MPTP and SML; *4*) mice given MPTP and SML containing L-Dopa (5 mg/kg); *5*) mice given MPTP and L-Dopa in solution (50 mg/kg). $^{*}p<0.05$, $^{**}p<0.01$ in comparison with *1*; $^{+}p<0.05$, $^{++}p<0.01$ compared with *2*.

and cholesterol (molar ratio of 7:3) in an L-Dopa solution (2.5 mg/kg). The L-Dopa content of the liposomes was 0.036 mol/mol phosphatidylcholine.

The mice were divided into five groups. Mice of group 1 (controls) were given intraperitoneal injections of 0.9% NaCl for 21 days, and mice of groups 2-5 were injected with MPTP for 7 days, after which MPTP was injected with 0.9% NaCl (group 2), SML suspension (500 mg/kg, in group 3), suspension of L-Dopa-containing SML (5 mg/kg, group 4), and L-Dopa solution (50 mg/kg, group 5). The SML suspension, L-Dopa-containing SML, and free L-Dopa (solution) were administered 1 h after MPTP once on day 7 and then twice daily from day 8 till day 21. A

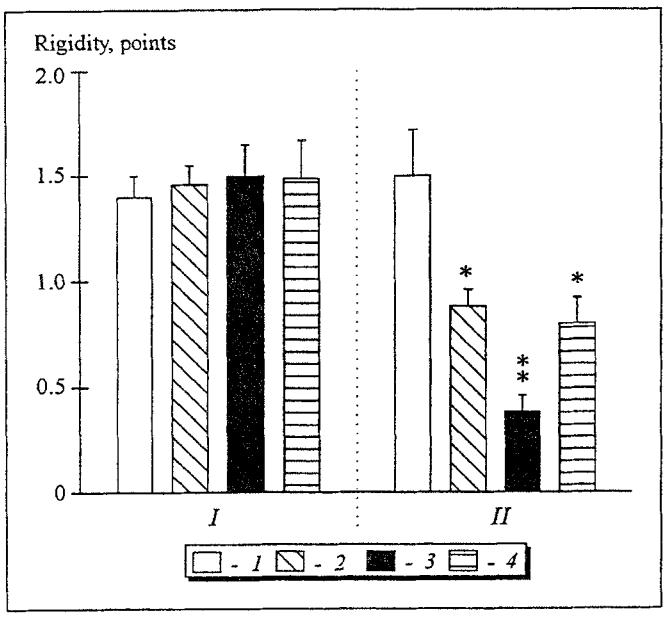

Fig. 2. Effects of free L-Dopa and L-Dopa-containing small monolamellar liposomes (SML) on the muscle rigidity in mice treated with MPTP for 7 (*I*) and 21 (*II*) days. *1*) mice given MPTP and 0.9% NaCl; *2*) mice given MPTP and SML; *3*) mice given MPTP and L-Dopa-containing SML; *4*) mice given MPTP and free L-Dopa. *$p<0.05$, **$p<0.01$ compared with *1*.

separate series of experiments was performed to assess the effect SML on the motor activity of intact mice: the animals were injected with SML according to the same scheme as mice with PS. Liposomes were injected intraperitoneally because their uptake by Kupffer cells and splenic macrophages in this case is 8-fold lower than that after intravenous administration [4]. A single dose of SML (500 mg/kg) was selected based on the maximum tolerated dose for mice [6].

The severity of PS was evaluated by the degree of muscle rigidity and oligokinesia. Muscle rigidity was scored in points [3]. The severity of oligokinesia was assessed by locomotor activity (the distance covered), vertical movements (the number of upright postures), and the time during which the mice did not move. These parameters were recorded using an Opto-Varimex-3 system (Columbus Instruments) [3] over a 3-min period on days 7, 14, and 21 of MPTP administration.

Statistical significance of intergroup differences was estimated by Student's *t* test, ANOVA test, and Neyman—Keuls post hoc test.

RESULTS

Before MPTP administration, there were no intergroup differences in motor activity (Fig. 1, *a-c*, *I*). On day 7 of MPTP administration, the distance covered was 2 times shorter, the period during which the mice did not move was 1.6 times longer, while the number of upright postures decreased by 7.7-fold compared with that in the control (Fig. 1, *a-c*, *II*); the rigidity was 1.5 points (Fig. 2, *I*). These changes in motor activity indicated the development of an akinetic-rigid form of PS by day 7 of MPTP administration.

The severity of PS was maximal on day 21 of MPTP administration, when the locomotor activity of MPTP-treated mice was 7.7-fold lower and the period during which they remained immobile was 2-fold longer than in the control group, while the number of upright postures was close to zero (Fig 1, *a-c*, *III*).

A single injection of SML with or without L-Dopa on day 7 of MPTP treatment had no effect on the severity of the PS. It should be noted that SML did not change the motor activity of intact mice. Analysis of motor activity after the last MPTP injection (day 21) in mice given SML without L-Dopa, L-Dopa-containing SML, or free L-Dopa starting from day 8 of MPTP administration, yielded the following results. The locomotor activity of mice given SML with and without L-Dopa 1 h after the last MPTP injection on day 21 increased 2- to 3-fold, while the period of immobility decreased 5-fold. Occasional upright postures were observed. In mice injected with free L-Dopa, clinical manifestations of PS disappeared almost completely (Fig. 1, *a-c*, *III*).

It is noteworthy that the motor activity of mice given L-Dopa-containing SML increased considerably 3 and 5 h after the last injection of MPTP (day 21). The locomotor activity increased 5-fold, the duration of immobility decreased 2.3-fold, and the number of upright postures increased 22-fold (Fig. 1, *a-c*, *IV* and *V*), while the muscle rigidity decreased (Fig. 2, *II*). Liposomes with L-Dopa completely inhibited clinical manifestations of PS by the 5th h after injection. By contrast, administration of free L-Dopa produced no appreciable effect on PS: after 3 h, motor activity of these mice remained practically the same as that of mice given MPTP and NaCl (Fig. 1, *a-c*, *IV*).

The motor activity of L-Dopa-treated mice increased on day 14 of MPTP administration; however, the increase was smaller than that observed on day 21.

It should be noted that a single injection of SML with L-dopa, unlike that of free L-Dopa, had no effect on the severity of PS. Therefore, it is likely that L-Dopa enclosed in SML gradually saturates the striatonigral structures as it is accumulated in the brain. Prolongation of the effects of L-Dopa-containing SML may be due to longer circulation of these liposomes in the bloodstream [6].

It should be stressed that prolongation of the therapeutic effect of L-Dopa enclosed in SML was achieved at a 10-fold lower concentration of this drug compared with that of free L-Dopa. In animals

with PS, SML may facilitate the delivery of L-Dopa to the brain and its accumulation in the striatonigral structures. Encapsulation into liposomes of some anticonvulsants (GABA, superoxide dismutase, and valproic acid) into liposomes increases their therapeutic effectiveness in rats with kindling [9,11,15]. However, the mechanisms by which liposomes transport drugs across the blood-brain barrier remain unknown. It was reported that liposomes 300 nm in diameter consisting of cholesterol and phosphatidylserine cross the blood-brain barrier after intravenous administration [14]. The diameter of liposomes used in this study was 60-80 nm as estimated by correlation laser spectroscopy.

Thus, L-Dopa-containing liposomes suppress clinical manifestation of Parkinson's syndrome more effectively than free L-Dopa, suggesting that they cross the blood-brain barrier providing effective saturation of the striatonigral structures with L-Dopa.

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