Effect of Long-Term Parenteral Administration of Empty and L-Dopa-Loaded Liposomes on the Turnover of Dopamine and Its Metabolites in the Striatum of Mice with Experimental Parkinson's Syndrome

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It is found that the dose of L-Dopa required for correction of dopamine metabolism in experimental Parkinson's syndrome in C57Bl/6 mice can be reduced 10-fold when the preparation is incorporated into small unilamellar liposomes (60 nm) composed from egg yolk phosphatidylcholine and cholesterol (7:3). High-performance liquid chromatography shows that a 14-day treatment with both L-Dopa incorporated into liposomes (5 mg/kg) and free L-Dopa (50 mg/kg) equally increases the content of dopamine and homovanillic acid and their ratio in mouse striatum. The content of dihydroxyphenylacetic acid markedly increases after administration of free L-Dopa.

Key Words: *Parkinson's syndrome; MPTP; L-Dopa-loaded liposomes; dopamine; striatum*

L-Dopa has an important role in substitutive therapy of Parkinson's disease [3,4]. However, the bulk of L-Dopa is rapidly excreted by the kidneys and decarboxylated in the blood and parenchymatous organs [1,4]. Correction of the preparation dose is very difficult due to disturbed dopamine storing in dopaminergic neuronal endings and denervation-induced hypersensitivity of dopamine receptors of the striatal neurons [3,11].

Incorporation into liposomes protects drugs from peripheral biodegradation, prolongs their lifetime in the circulation, and reduces their toxicity [7]. We have demonstrated that administration of low doses of L-Dopa (5 mg/kg) incorporated into small monolamellar liposomes (SML) produces a prolonged positive effect on motor disturbances in mice with Parkinson's syndrome (PS) induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [13]. In the present study we examined the effects of empty and L-Dopa-loaded SML on the content of dopamine and its metabolites in the striatum of mice with experimental PS.

MATERIALS AND METHODS

Experiments were performed on C57Bl/6 male mice weighing 23-25 g. PS was induced by MPTP injected intraperitoneally in a dose of 20 mg/kg, twice per day at 12-h intervals for 22 days [5]. SML were

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prepared in 0.9% NaCl by sonication of a suspension of multilamellar liposomes consisting of egg yolk phosphatidylcholine and cholesterol (7:3 mole ratio, lipid concentration 50 mg/ml) in a solution containing 2.5 mg/ml L-Dopa [15]. L-Dopa not incorporated into liposomes was removed by equilibrium dialysis against 0.9% NaCl. Laser correlation spectroscopy [6] showed that the size of 90% of sonicated liposomes ranged within 20-30 nm. Pharmacological intervention was performed from days 8 through 22 of the experiment twice a day with 12-h intervals. Empty and L-Dopa-loaded SML were injected intraperitoneally in a dose of 500 mg lipid/kg body weight, the dose of L-Dopa being 5 mg/kg (in liposomes) and 50 mg/kg (in solution). The control animals received 0.9% NaCl instead of MPTP and L-Dopa. In order to evaluate the effect of SML on dopamine metabolism in mice without PS, SML were injected according to the same scheme. On day 22, the animals were decapitated at 10:00-12:00. The brain was removed, and the striatum was isolated at 0-4°C. The content of dopamine and its metabolites (dihydroxyphenylacetic acid, DOPAC, and homovanillic acid, HVA) in the striatum was measured by high-performance liquid chromatography with electrochemical detection [9].

The data were processed statistically, and the significance of differences was evaluated by Student test and by ANOVA using the Neyman—Keuls post hoc test.

RESULTS

In mice with PS, the content of dopamine and its metabolites DOPAC and HVA in the striatum dropped by 90, 78, and 80%, respectively (Table 1). Injection of empty SML into animals with PS did not increase the dopamine concentration, while administration of both L-Dopa in SML and free L-Dopa equally increased the content of dopamine 1.8- to 2-fold (Fig. 1, *a*, *I*). Injection of SML elevated the content of DOPAC 1.8-fold (Fig. 1, *a*, *II*) without parallel rise of the DOPAC/dopamine ratio (Fig. 1, *b*, *I*). The effect of free L-Dopa on the DOPAC content was more pronounced than that of L-Dopa incorporated into SML (Fig. 1, *a*, *II*), whereas the maximum rise of the DOPAC/dopamine ratio was produced by L-Dopa-loaded SML (Fig. 1, *b*, *I*). The content of HVA increased 2.8-fold after administration of SML and 3.3-fold after injection of free L-Dopa (Fig. 1, *a*, *III*). L-Dopa-loaded SML produced an intermediate effect (Fig. 1, *a*, *III*). The HVA/dopamine ratio increased in all groups of the mice injected with the preparations, attaining the maximum in the SML-treated group (Fig. 1, *b*, *II*). The total content of dopamine and its metabolites in animals injected with empty SML, L-Dopa-loaded SML, and free L-Dopa increased by 71, 104, and 277%, respectively (Fig. 1, *b*, *III*).

The changes induced by injection of SML were similar in mice given physiological saline and in mice with MPTP-induced PS. The absolute content of HVA and HVA/dopamine ratio increased 1.3- and 1.5-fold compared with the control (Table 1). The total content of dopamine, HVA, and DOPAC increased by 45% (Table 1).

It can be hypothesized that empty SML of the above-mentioned composition have similar effects on metabolic transformation of dopamine to HVA in intact animals and in mice with PS (Fig. 1, Table 1).

This hypothesis is supported by increase in the HVA content (Fig. 1, *a*, *III*) and the HVA/dopamine ratio (Fig. 1, *b*, *II*). Previously, it was shown that intravenous injection of liposomes consisting of egg yolk phosphatidylcholine and phosphatidylserine promotes catecholamine release [10], activates dopamine-sensitive adenylate cyclase, and increases cAMP content in mouse striatum [12]. Thus, enhanced transformation of dopamine into HVA in the striatum may result from its enhanced secretion followed by extraneuronal metabolism catalyzed by catechol-O-methyltransferase [2,11]. Injection of empty SML had no or little effect on the level of DOPAC and the DOPAC/dopamine ratio, which characterize the

TABLE 1. Content of Dopamine and Its Metabolites in the Striatum of C57Bl/6 Mice After Long-Term Administration of SML and in MPTP-Induced Experimental Parkinson's Syndrome (*M±m*)

Parameter	Control (*n*=15)	SML (*n*=12)	PS (*n*=10)
Dopamine, ng/mg	3.42±0.51	3.29±0.41	0.386±0.085**
DOPAC, ng/mg	0.35±0.07	0.38±0.07	0.077±0.022**
HVA, ng/mg	1.37±0.07	1.63±0.09*	0.27±0.037**
Dopamine+DOPAC+HVA, pmol/mg	22.6±2.1	32.2±2.24*	4.5±0.6**
DOPAC/dopamine, %	10±1.6	13±3.5	22±6*
HVA/dopamine, %	37±7.2	54±3.2*	60±12.4

Note. *$p<0.05$, **$p<0.001$ in comparison with the control.

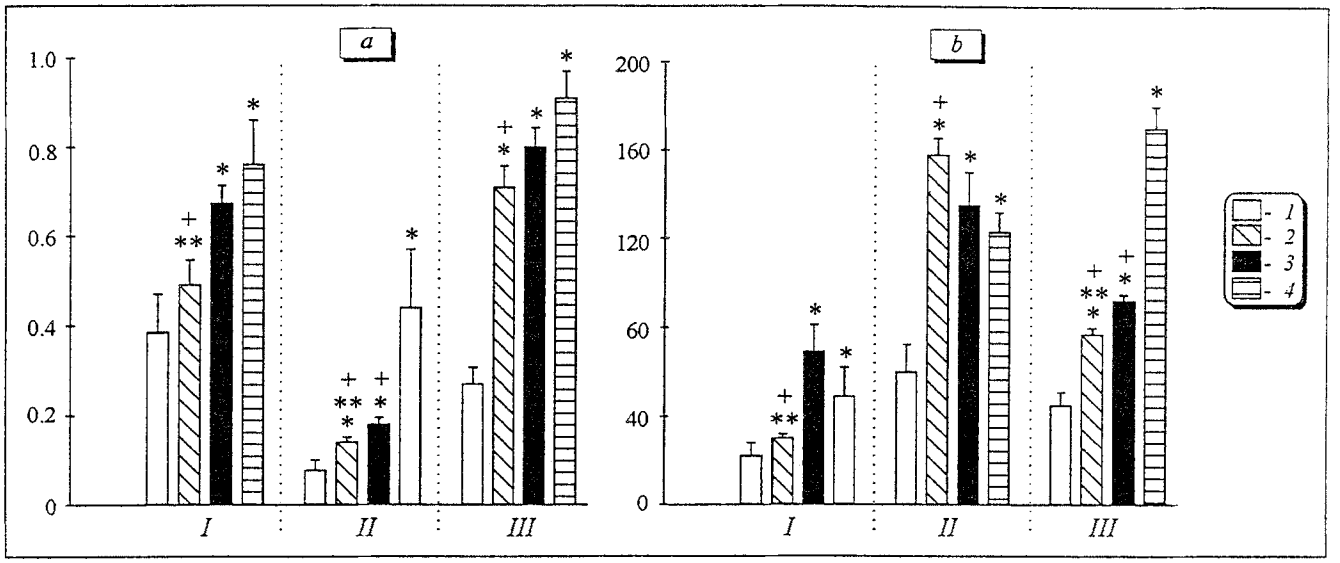

Fig. 1. Effect of L-Dopa-loaded SML on the content of dopamine and its metabolites in the striatum of mice with Parkinson's syndrome on day 21 of MPTP treatment. *1*) NaCl; *2*) SML; *3*) L-Dopa-loaded SML (5 mg/kg); *4*) L-Dopa (50 mg/kg). *a*: *I*) dopamine; *II*) DOPAC; *III*) HVA, ng/mg tissue; *b*: *I*) DOPAC/dopamine, %; *II*) HVA/dopamine, %; *III*) dopamine+DOPAC+HVA, mol×10^{-13}/mg tissue. $p<0.05$: *in comparison with *1*; **in comparison with *3*; +in comparison with *4*. Each group comprised 10-12 animals.

intracellular metabolism of dopamine into DOPAC catalyzed by monoamine oxidase [1,2,11]. This suggests that SML stimulate dopamine secretion from neuronal endings in the striatum and, probably, retard its biodegradation in the presynaptic endings. However, the observed increase in the total content of dopamine and its metabolites produced by SML (Table 1, Fig. 1, *b*, *III*) suggests that SML may enhance dopamine synthesis in preserved neurons. A possible mechanism of this phenomena is as follows: lipids constituting the liposomes incorporate into the plasma membrane and modulate the conformation of lipid-protein complexes and receptors regulating dopamine synthesis and secretion [8,11]. Both L-Dopa-loaded SML and free L-Dopa increased the dopamine content in the striatum to the same extent despite the fact that the dose of L-Dopa in liposomes was 10-fold lower (Fig. 1, *a*, *I*). This discrepancy is probably associated with the fact that the bulk of free L-Dopa undergoes natural biotransformation catalyzed by peripheral Dopa-decarboxylase, and no more than 20% of the injected dose is delivered into the brain [1,11]. On the other hand, under conditions of dopamine deficiency in the striatum in PS, if large quantities of L-Dopa are simultaneously delivered into the brain and rapidly decarboxylated to dopamine, part of the newly synthesized neurotransmitter is transformed by monoamine oxidase into DOPAC and does not perform its synaptic function [1,11]. This suggestion is indirectly confirmed by the fact that the absolute content of DOPAC in the striatum rose only after injection of free L-Dopa but not L-Dopa-loaded SML (Fig. 1, *a*, *II*). By contrast, the intensity of DOPAC generation (DOPAC/dopamine ratio) reached the maximum in animals injected with L-Dopa-loaded SML (Fig. 1, *b*, *I*). This presumably results from gradual delivery of L-Dopa-loaded SML to the brain due to their prolonged circulation in the blood, which is typical of drugs incorporated into liposomes [7]. Both L-Dopa-loaded SML and free L-Dopa had the same effect on the extracellular level of dopamine released from nerve endings [2,4], the level of HVA, and the intensity of its formation (HVA/dopamine ratio) (Fig. 1, *b*, *II*). However, when evaluating these shifts, the above-mentioned effect of liposome-derived lipids on the formation of HVA from dopamine should be taken into account. The rise in the total content of dopamine and its metabolites induced by L-Dopa-loaded SML (Fig. 1, *b*, *III*) suggests that the observed changes in dopamine metabolism result from the delivery of exogenous L-Dopa to the nigrostriatal structures.

Thus, study of dopamine metabolism in mice with PS showed that long-term administration of L-Dopa-loaded SML has no effect on the content of DOPAC in the striatum and induces the same shifts in the content of dopamine and HVA as does free L-Dopa administered through the same route but in a 10-fold higher dose. It was also found that empty SML composed from egg yolk phosphatidylcholine and cholesterol modulate the formation of HVA from dopamine both in healthy mice and in mice with experimental PS. Moreover, our studies demonstrated that the dose of L-Dopa required for correction of dopamine metabolism in experimental PS can be reduced 10-fold when SML are used as a vehicle.

Our findings suggest that liposomes are able to protect L-Dopa from peripheral decarboxylation. On the other hand, the blood-brain barrier is probably not an absolute obstacle for L-Dopa-loaded liposomes. However, the questions of whether SML are able to facilitate the crossing of the blood-brain barrier for L-Dopa, and whether SML or their components enter the brain, require special investigations.

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