# GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

# Effects of L-Dopa-Carrying Liposomes on Striatal Concentration of Dopamine and Its Metabolites and Phospholipid Metabolism in Experimental Parkinson's Syndrome

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Intraperitoneal injection of extremely low doses of L-Dopa-containing liposomes increases the rate of dopamine metabolism and alters the metabolism of signal phospholipids in the striatum of C57Bl/6 mice with experimental parkinsonism. Liposomal lipids affect the phospholipid metabolism in these mice.

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

The blood-brain barrier shows a high selectivity for substances passing from the blood into the central nervous system. It was demonstrated that drugs encapsulated into liposomes are capable of crossing this barrier [10,12]. Encapsulation into liposomes alters pharmacokinetics, decreases toxicity, and protects the drug from premature biodegradation [7]. So far, it remains unclear whether liposomes by themselves can pass though the blood-brain barrier [10, 12]. In this study we evaluated the ability of L-Dopa-containing liposomes to affect the metabolism of dopamine, dopamine derivatives, and phospholipids in the striatum of mice with experimental parkinsonism.

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## MATERIALS AND METHODS

C57Bl/6 mice weighing 23-25 g were used. The Parkinson's syndrome (PS) was induced by intraperitoneal injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a dose of 20 mg/kg body weight 2 times daily at a 12-h interval during a 7-day period [2]. Intact mice (control) received normal saline by the same scheme. The development of PS was confirmed by changes in the major parameters of motor activity in mice, such as the distance covered and the durations of rest and movement [4]. The parameters were measured using an Opto-Varimex-3 system (Columbus Instruments). On day 8, the mice with PS received an intraperitoneal injection of 1) normal saline, 2) a suspension of L-Dopa-containing liposomes (5 mg) in a dose of 500 mg lipids/kg body weight, or 3) a suspension of "empty" liposomes in the same dose. The volume of injection was 0.2 ml.

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**TABLE 1.** Dopamine and Dopamine Metabolite Levels (ng/mg Tissue) and Dopamine Turnover (rel. units) in the Striatum of C57Bl/6 Mice with PS After a Single Injection of Liposomes Loaded with L-Dopa ($M\pm m$; $n$=8-10 per group)

| Group | Dopamine | DHPAA | HVA | DHPAA/ dopamine | HVA/ dopamine | DHPAA+HVA/ dopamine |
|---|---|---|---|---|---|---|
| Control | 5.92±1.4 | 0.96±0.13 | 1.3±0.1 | 0.24±0.09 | 0.39±0.2 | 0.30±0.02 |
| MPTP | 0.97±0.2** | 0.27±0.04*** | 0.5±0.1*** | 0.31±0.05 | 0.51±0.04*** | 0.71±0.03*** |
| MPTP+L-Dopa-containing liposomes | 0.69±0.2** | 0.28±0.03*** | 0.5±0.14** | 0.96±0.13*,+ | 0.92±0.16**,+ | 1.81±0.36*,+ |
| MPTP+L-Dopa-free liposomes | 0.81±0.1** | 0.24±0.07*** | 0.36±0.03*** | 0.21±0.02+ | 0.38±0.07+ | 0.53±0.06*,+ |

**Note.** *$p$<0.05, **$p$<0.01, ***$p$<0.001 compared with control mice; +$p$<0.05 compared with MPTP-treated mice. DHPAA = dihydroxyphenylalanineacetic acid; HVA = homovanillic acid.

One hour after injection, the mice were decapitated, the brain was removed, and the striatum was isolated at 0-4°C, rapidly frozen, weighed, and stored in liquid nitrogen.

Small unilamellar liposomes were prepared by sonication of multilamellar liposomes consisting of egg yolk phosphatidylcholine and cholesterol (molar ratio 7:3, total lipid concentration 50 mg/ml) in normal saline containing 2.5 mg/ml L-Dopa. The suspension was sonicated 10 times at 1-min intervals (30 sec each time) in a UZDN-2T apparatus at 22 kHz. Metal particles were separated by centrifugation at 3000$g$ for 6 min [13]. Unincorporated L-Dopa was separated by dialysis against 0.9% NaCl. The liposome diameter was 60-100 nm, and the mean L-Dopa content was 0.053±0.006 mol/mol phosphatidylcholine.

The contents of dopamine and its metabolites in the striatum were measured by high-performance liquid chromatography with electrochemical detection [3].

Lipids were extracted from tissues by the method of Folch. Phospholipids were separated by thin-layer chromatography. The following fractions were obtained: lysophospholipid, sphingomyelin, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and phosphatidic acids. Each fraction was then transferred into a thermostable test tube and treated with $HClO_4$ for 40 min at 190°C. An aliquot of the total lipid extract was also treated in the same way after evaporation. Inorganic phosphorus was measured after decomposition [5], and the results were expressed in nmol phosphorus/mg tissue. Reagents were from Serva. Salts were from Merck.

The data were statistically analyzed. The significance of differences was evaluated by Student's $t$ test.

## RESULTS

MPTP strongly inhibited the motor activity of mice: the mean covered distance was 160±96 cm *vs.* 615±57 cm in the control ($p$<0.01), the mean time of movement was 8±3 sec *vs.* 49±4 sec ($p$<0.01), while the mean resting time was 144±7 sec *vs.* 61±5 sec ($p$<0.01). In other words, the animals treated with this neurotoxin developed oligokinesia characteristic of the PS.

The concentrations of dopamine and its metabolites dihydroxyphenylalanineacetic acid (DHPAA) and homovanillic acid (HVA) in the striatum of mice with PS were reduced by 80, 70, and 60%, respectively (Table 1). The concentrations of dopamine and its metabolites in mice treated with L-Dopa-carrying or L-Dopa-free liposomes did not differ considerably from those in the control.

In mice with PS, the mean parameters of dopamine turnover: the concentration ratios of dopamine metabolites to dopamine (HVA/dopamine, DHPAA/dopamine, and DHPAA+HVA/dopamine), were higher than in the control group (Table 1), indicating a compensatory enhancement of dopamine metabolism in animals with PS [11].

A tendency toward a decrease in the DHPAA/dopamine and HVA/dopamine ratios and a statistically significant reduction in the DHPAA+HVA/dopamine ratio were observed in MPTP-treated mice injected with liposomes in comparison with mice given MPTP alone (Table 1). In mice injected with L-Dopa-containing liposomes, the dopamine turnover was significantly higher than in other mice.

The total content of phospholipids in the striatum of mice with PS treated and untreated with liposomes did not differ significantly from that in the control group (Table 2). In mice with PS, the striatal contents of sphingomyelin, phosphatidylcholine, phosphatidylserine, lysophospholipid, and phosphatidylethanolamine practically did not change, while the phosphatidylinositol content tended to increase. Injection of L-Dopa-free liposomes into mice with PS did not alter the striatal content of lysophospholipid or phosphatidylserine.

Injection of L-Dopa-containing lyposomes had no effect on the striatal content of phosphatidylserine and slightly increased that of lysophospholipid. In

**TABLE 2.** Phospholipid Levels (nmol/mg Tissue) in the Striatum of C57Bl/6 Mice with PS After a Single Injection of Liposomes Loaded with L-Dopa ($M \pm m$; $n$=8-10 per group)

| Group | Phospho-lipids | Lisophospho-lipids | Sphingo-myelin | Phosphatidyl-choline | Phosphatidyl-inositol | Phosphatidyl-serine | Phosphatidyl-ethanolamine | Phosphatidic acids |
|---|---|---|---|---|---|---|---|---|
| Control | 59.2±4.24 | 0.26±0.08 | 2.39±0.48 | 20.40±1.60 | 3.43±0.34 | 6.33±0.50 | 19.39±2.56 | 7.19±0.22 |
| MPTP | 58.2±1.33 | 0.25±0.04 | 2.06±0.04 | 19.89±0.87 | 3.93±0.15 | 6.60±0.37 | 17.11±0.64 | 8.87±0.45*** |
| MPTP+L-Dopa-containing liposomes | 54.8±3.6 | 0.37±0.07 | 1.58±0.05++ | 17.64±1.03 | 3.05±0.28+ | 5.67±0.58 | 13.95±2.35 | 12.35±0.97+,*** |
| MPTP+ L-Dopa-free liposomes | 56.2±1.53 | 0.23±0.02 | 1.55±0.15+ | 16.52±0.39+ | 2.91±0.21++ | 6.03±0.37 | 13.93±0.41+++ | 8.95±0.60*** |

**Note.** *$p$<0.05, **$p$<0.01, ***$p$<0.001 compared with control mice; +$p$<0.05, ++$p$<0.01, +++$p$<0.001 compared with MPTP-treated mice.

mice with PS treated with L-Dopa-free liposomes, the striatal contents of sphingomyelin, phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine decreased. In mice with PS treated with L-Dopa-containing liposomes, the striatal contents of sphingomyelin and phosphatidylinositol decreased, the levels of phosphatidylcholine and phosphatidylethanolamine showed a tendency toward a decrease, and the phosphatidylinositol level returned to normal. Special attention should be paid to phosphatidic acids. Their content was elevated in the striatum of mice with PS. After injection of L-Dopa-containing liposomes, the content of phosphatidic acids increased considerably compared with both control and PS mice. In the striatum of mice with PS treated with L-Dopa-free liposomes, the phosphatidic acid concentration was higher only in comparison with the control (Table 2).

Taken together with the findings of other researchers [15], our results show that MPTP lowers dopamine and dopamine metabolite levels in the striatum. There was no absolute rise in the striatal concentration of dopamine or its metabolites one hour after a single injection of liposomes with and without L-Dopa. An increase in the rate of dopamine turnover in mice with PS may result from a compensatory stimulation of dopamine metabolism. A single injection of L-Dopa-free liposomes into mice with PS led to a slight decrease in the rate of dopamine metabolism. Presumably, this is due to the effect of liposomal phosphatidylcholine on the dopamine metabolism: phosphatidylcholine serves as a source of choline, which is utilized in the synthesis of acetylcholine, a compound stimulating dopamine metabolism in the striatum via M-cholinergic receptors [6]. On the other hand, injection of the L-Dopa-containing liposomes markedly increased the rate of dopamine metabolism, suggesting that L-Dopa is delivered to the CNS where it is metabolized to dopamine, DHPAA, and HVA. The small size of liposomes prevented their uptake by cells of the reticuloendothelial system. Previously, we found that liposomes of the same composition retain L-Dopa for 14 days.

There are several explanations for the fact that the levels of dopamine and its metabolites did not increase despite a high rate of dopamine metabolism. First, the dose of L-Dopa administered in liposomes was 10-fold lower than that traditionally used for correcting parkinsonism in animals [14]. This is due to a low efficiency of L-Dopa incorporation into liposomes and limitations of the dose of injected lipids. Second, since the rate of L-Dopa metabolism in the brain is very high, the newly formed dopamine performs the synaptic function and is rapidly con-

verted to DHPAA and HVA [11]. Therefore, an increase in the rate of dopamine metabolism may be attributed to the supply of the striatonigral structures with L-Dopa from an external source, namely, L-Dopa-containing liposomes. Third, L-Dopa can act as a neurotransmitter, modulating the release of dopamine in amounts 3- to 4-fold higher than those released when L-Dopa is used as a precursor of dopamine [8].

Phospholipid metabolism in nerve tissue depends on its functional activity. An increase in the striatal contents of phosphatidylinositol and phosphatidic acids in mice with PS is probably associated with the second messenger system which is involved in the regulation of neurotransmitter secretion in the striatum. Normalization of phosphatidylinositol levels by liposomes with or without L-Dopa may be ascribed to the effect of liposomal lipids on the second messengers. An increase in the concentrations of lysophospholipid and phosphatidic acid may be directly associated with the activity of L-Dopa delivered by liposomes. Dopamine activates cAMP-dependent protein kinase which stimulates phospholipase $A_2$ [9]. This may account for the substantially increased lysophospholipid production after administration of L-Dopa-containing liposomes. It is likely that the pronounced increase in the phosphatidic acid concentration in L-Dopa-treated mice is due to the ability of dopamine to modify the phosphoinositide metabolism via $D_2$ receptors [1]. On the other hand, since the striatum contains cholinergic, GABAergic, enkephalinergic, and other neurons, alterations in the metabolism of signal phospholipids can be regarded as the total effect of interneuronal interactions occurring after injection of L-Dopa-containing liposomes. Variations in major membrane phospholipids (sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine) observed after injection of liposomes with and without L-Dopa were similar, resulting probably from accelerated turnover of these phospholipids after administration of an essentially high dose of lipids.

Thus, changes in dopamine and phospholipid metabolism after injection of low doses of L-Dopa can be attributed to the ability of liposomes to protect L-Dopa from degradation and provide its passage through the blood-brain barrier. Liposomal lipids by themselves influence the phospholipid metabolism in the striatum, suggesting that the liposome membrane components have reached the brain.

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