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**Rapid communication**

# An application of the microdialysis system to the pharmacokinetic study on striatal distribution of L-DOPA with or without carbidopa in rats

Mikiro Nakashima, Mihoko Nakano, Kenji Matsuyama and Masataka Ichikawa

*Department of Hospital Pharmacy, School of Medicine, Nagasaki University, 7-1 Sakamoto-machi, Nagasaki 852 (Japan)*

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**Summary**

The effect of coadministration of carbidopa on the contents of L-DOPA and its active metabolite, dopamine, in the rat striatum after the intraperitoneal administration of L-DOPA was investigated by the use of the microdialysis system, employing a statistical moment analysis. It was demonstrated that the contents of L-DOPA and dopamine in the striatum were significantly increased by 7.8 and 3.3 times when carbidopa was coadministered, respectively.

From the present study, the microdialysis system is found to be a suitable technique to assess the distribution of drugs in the specific brain region in in vivo state.

The microdialysis system is a newly developed brain perfusion technique in which a semi-permeable membrane cannula perfused with a physiological solution is stereotaxically implanted into a selected brain region according to a brain map (Ungerstedt et al., 1982; Zetterström et al., 1983).

An important advantage of this technique is that it makes possible to measure endogenous chemicals, e.g., catecholamines (Zetterström et al., 1983), amino acids (Tossman and Ungerstedt, 1986) and acetylcholine (Consolo et al., 1987; Damsma et al., 1987), released in the extracellular space in various brain regions without excision of brain.

In the present study, we have tried to apply the microdialysis system to the pharmacokinetic study of drugs capable of penetrating the blood-brain barrier (BBB). As a model drug, L-dihydroxyphenylalanine (L-DOPA), the amino acid precursor of dopamine (DA), was chosen and its distribution in the striatum was examined with or without carbidopa, the aromatic L-amino acid decarboxylase inhibitor, by the use of the microdialysis system, employing a statistical moment analysis (Cutler, 1978; Yamaoka et al., 1978).

L-DOPA and DA were purchased from Sigma (St. Louis, MO). Carbidopa was a gift from Banyu

*Correspondence*: Masataka Ichikawa, Department of Hospital Pharmacy, School of Medicine, Nagasaki University, 7-1 Sakamoto-machi, Nagasaki 852, Japan.

Pharm. (Tokyo). All other chemicals were of special reagent grade. Male Wistar rats (SPF), weighing 280–320 g, were anaesthetized with urethane (1.5 g/kg as 300 mg/ml, i.p.) and mounted in a stereotaxic instrument. A dialysis probe with the dialysing membrane length of 3 mm and the outer diameter of 0.5 mm (CMA/10, Carnegie Medicin, Stockholm) was inserted into the striatum (coordinates: rostral, +0.7 mm; lateral, +2.5 mm; ventral, −6.5 mm, relative to bregma and the dura surface) according to the Paxinos and Watson atlas (1986). The probe was connected to a microinjection pump (CMA/100, Carnegie Medicin) and continuously perfused with Ringer's solution (147 mEq/l $Na^+$, 4.5 mEq/l $Ca^{2+}$, 4 mEq/l $K^+$ and 155.6 mEq/l $Cl^-$; pH 6.5) at a rate of 2 μl/min. Perfusates collected over the first 100 min following the probe insertion were discarded to obtain stable levels of endogenous DA and its related compounds. Thereafter, three dialysate samples from 100 to 160 min after the probe insertion were collected to obtain the stable baseline levels of DA and its related compounds before the administration of L-DOPA. In the case of administration of L-DOPA alone, at 160 min after the probe insertion, control vehicle (an acidified saline) was intraperitoneally administered, and subsequently 40 min later L-DOPA was administered intraperitoneally at a dose of 250 μmol/kg. In the case of coadministration of carbidopa, carbidopa was first administered at a dose of 25 μmol/kg and subsequently 40 min later L-DOPA was administered intraperitoneally at a dose of 250 μmol/kg. In the case of controls, rats received vehicle only twice in a similar manner as the administration of L-DOPA and carbidopa. L-DOPA and carbidopa were dissolved in a vehicle solution at a concentration of 100 μmol/ml and 25 μmol/ml, respectively. The dialysate sample was collected into a small sample tube containing 10 μl of 1 M perchloric acid at every 20 min interval up to 6 h after the administration of L-DOPA.

We used a LC-6A pump (Shimadzu, Kyoto) equipped with a L-ECD-6A detector (Shimadzu) and a Chromatopac C-R3A recorder (Shimadzu) in order to determine the content of L-DOPA, its metabolites and carbidopa. For detection, a electrochemical detector was used, set at +800 mV. The separation was performed on a TSK-GEL ODS-80TM column (Tosoh, Tokyo; 150 × 4.6 mm, i.d., particle size 5 μm). The elution was performed at a flow rate of 1.0 ml/min at room temperature by using a mobile phase consisting of 0.05 M potassium dihydrogen phosphate buffer (pH 2.95, adjusted with phosphoric acid) containing 1.1 mM sodium octanesulfonate, 1 mM disodium EDTA and 15% methanol.

The data were evaluated by moment analysis. The first three (zero-th to second) statistical moment parameters (moments) are difined as follows:

$$X(0\text{–}6\ h) = \int_0^6 \left( \frac{d(X - X_b)}{dt} \right) dt \qquad (1)$$

$$\mathrm{MRT} = \int_0^6 t \left( \frac{d(X - X_b)}{dt} \right) dt / X(0\text{–}6\ h) \qquad (2)$$

$$\mathrm{VRT} = \int_0^6 (t - \mathrm{MRT})^2 \left( \frac{d(X - X_b)}{dt} \right) dt / X(0\text{–}6\ h) \qquad (3)$$

where $t$ is time, $X$ is the amount of the substance in the dialysate and $X_b$ is the endogenous baseline level of the substance in the dialysate. $X$(0–6 h), MRT and VRT are the total recovery of the substance in the dialysate from 0 to 6 h after the administration of L-DOPA, the mean residence time of the substance in the striatum, and the variance of the MRT, respectively.

The time course of the amounts of L-DOPA in dialysates collected from the striatum are shown in Fig. 1.

It is obvious that coadministration of carbidopa gives rise to a significant increase of the amounts of L-DOPA in the striatal dialysates. On the basis of the time course data, moment analysis was performed and the results are shown in Table 1.

The total recovery of L-DOPA from 0 to 6 h was 9.02 pmol when L-DOPA was administered alone. On the other hand, the coadministration

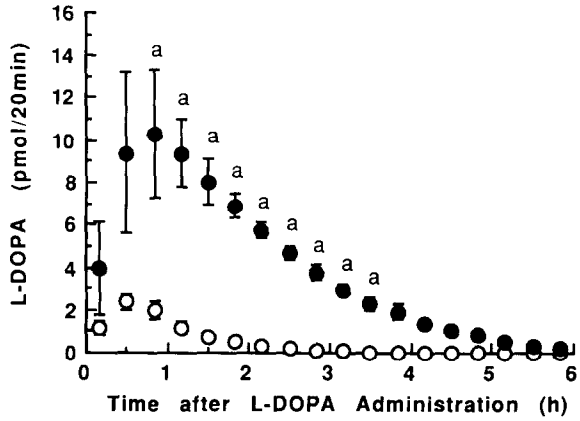


Fig. 1. Time course of the amounts of L-DOPA in the dialysates collected from the striatum after intraperitoneal administration of L-DOPA with (●) or without (○) carbidopa in the rat. Points represent the mean ± S.D. of 5 rats. a Points are significantly different from the carbidopa (–) at $p < 0.001$ when assessed by Student's $t$-test.

of L-DOPA and carbidopa resulted in a significant increase in the total recovery of L-DOPA by 7.8 times (70.6 pmol). MRT and VRT calculated from the above time course data were significantly increased by 1.7 and 2.4 times in the case of coadministration of L-DOPA and carbidopa, respectively.

When vehicle was administered as a control, the amounts of DA in the striatal dialysates were the same as an endogenous baseline level of DA. The intraperitoneal administration of L-DOPA resulted in a significant increase in the amounts of DA compared to the control amounts. The total recovery of DA from 0 to 6 h was 0.630 pmol which is thought to be equivalent to the amounts of metabolite for L-DOPA penetrating the BBB (Table 1).

Furthermore, in the case of coadministration of carbidopa, the amounts of DA were significantly increased and the value of the total recovery was increased by 3.3 times (2.05 pmol) as much as in the case of administration of L-DOPA alone (Table 1).

In the present study, the significant increases of the contents of L-DOPA and its active metabolite, DA, in the striatum following the administration of L-DOPA with carbidopa are successfully observed in the in vivo state using the microdialysis system. It is suggested that the microdialysis system is a suitable technique for the examination of the distribution of drugs in a specific brain region.

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TABLE 1

*Moment parameters of L-DOPA and DA in the striatum after intraperitoneal administration of L-DOPA with or without carbidopa in the rat*

| Compound | Carbidopa | Moment parameters [a] | | |
|---|---|---|---|---|
| | | $X$(0–6 h) (pmol) [b] | MRT (h) | VRT (h) |
| L-DOPA | – | 9.02 ± 1.47 | 1.04 ± 0.11 | 0.591 ± 0.204 |
| | + | 70.6 ± 11.2 [c] | 1.81 ± 0.20 [c] | 1.44 ± 0.16 [c] |
| DA | – | 0.630 + 0.195 | 1.87 ± 0.42 | 0.788 ± 0.358 |
| | + | 2.05 ± 0.17 [c] | 2.24 ± 0.34 | 1.47 ± 0.66 |

Rats received a dose of 250 μmol/kg of L-DOPA with or without a dose of 25 μmol/kg of carbidopa.

[a] Values represent the mean ± S.D. of 5 rats.

[b] Total amounts of compound collected in the dialysates from 0 to 6 h.

[c] Values are significantly different from the carbidopa (–) at $p < 0.001$ when assessed by Student's $t$-test.

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