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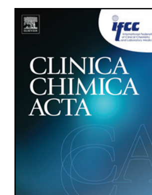

Noninvasive monitoring of plasma L-dopa concentrations using sweat samples in Parkinson's disease

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ABSTRACT

Background: L-dopa (L-3,4-dihydroxyphenylalanine) is commonly used for treating Parkinson's disease (PD). However, regardless of its prominent effect, therapeutic range of L-dopa narrows down with disease progression, which leads to development of motor complications including wearing off and dyskinesias. In addition, intestinal absorption of L-dopa is inversely correlated with the amount of oral protein intake, and shows intra- and inter-day variability. Hence, frequent monitoring of plasma L-dopa concentrations is beneficial, but frequent venipuncture imposes physical and psychological burdens on patients with PD.
Methods: We investigated the usefulness of sweat samples instead of plasma samples for monitoring L-dopa concentrations. With a monolithic silica disk-packed spin column and the high-performance liquid chromatography-electrochemical detection system, L-dopa in sweat samples was successfully quantified and analyzed in 23 PD patients.
Results: We found that the Pearson's correlation coefficient of the plasma and sweat L-dopa concentrations was 0.678. Although the disease durations and severities were not correlated with the deviation of the actual sweat L-dopa concentrations from the fitted line, acquisition of the sweat samples under a stable condition was technically difficult in severely affected patients. The deviations may also be partly accounted for by skin permeability of L-dopa.
Conclusions: Measuring L-dopa concentrations in sweat is suitable to get further insights into the L-dopa metabolism.

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1. Introduction

Parkinson's disease (PD) is a progressively disabling neurodegenerative disorder with clinical manifestations of bradykinesia, tremor, rigidity, and postural instability. PD is pathologically characterized by the loss of dopaminergic neurons in the substantia nigra in the midbrain. In PD, the course of the clinical decline parallels that of the progressive degeneration of the dopaminergic neurons [1]. Levodopa, which is an INN nomenclature for L-dopa, is widely used as the standard drug for PD, because L-dopa is highly effective as the dopamine-replacement therapy in ameliorating the symptoms in PD [2]. However, in the course of long-term L-dopa treatment and of disease progression, the duration of the drug effect becomes short and motor complications, notably "dyskinesias" and "wearing-off", start to appear [3].

Two major strategies are currently used to suppress the development of motor complications in the later stages of PD. In the first strategy, long-acting dopamine receptor agonists are administered from an early stage of PD [4]. The dopamine receptor agonists are able to stimulate the striatal dopamine receptors continuously, which is likely to be the basis for the suppression of the development of motor complications. However, the dopamine receptor agonists are not as effective as L-dopa in ameliorating the PD symptoms and addition of L-dopa becomes inevitable in the course of progression of PD in most patients [5]. The second strategy focuses on the fact that L-dopa is a short-acting drug with a plasma half-life ($t_{1/2}$) of 1.5 h even when combined with a decarboxylase inhibitor like benserazide or carbidopa, the marked fluctuation of the plasma L-dopa levels provokes motor complications with disease progression. To extend the effective duration of orally administered L-dopa, L-dopa is administered with a COMT inhibitor such as entacapone. Early treatment of PD with L-dopa and entacapone results in a lower incidence of "wearing-off", but its effect on "dyskinesia" remains elusive [6].

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The striking effect of L-dopa makes the L-dopa therapy the gold standard for treating PD, although marked fluctuation of plasma L-dopa levels is unavoidable. These peculiarities of L-dopa pose problems to the conventional approach of therapeutic drug monitoring, i.e., the relation between blood concentrations and clinical and/or toxicological effects [7]. Frequent blood sampling, however, imposes physical and psychological burdens on PD patients.

Sweat can be used as an alternative sample source since it is easily and noninvasively accessible without burdening the patients. Three representative kinds of sweating are known in our bodies: thermoregulatory sweating, emotional sweating, and insensible perspiration. The main constitutes of sweat are water and high concentrations of sodium and chloride.

2. Methods

2.1. Chemicals

L-Dopa, norepinephrine, epinephrine, and dopamine were from Sigma. Acetonitrile (HPLC grade) was from Wako Pure Chemical. Purified water was prepared using a Millipore ultra-pure water system.

2.2. Human plasma and sweat samples

All studies involving human subjects were approved by the ethical review committees of the Nagoya University Graduate School of Medicine (and the Graduate School of Pharmaceutical Sciences, University of Tokyo). After appropriate informed consents were obtained, blood (5 ml) was drawn from 23 PD patients taking L-dopa and 3 normal controls. After isolation of plasma, all plasma samples were kept at −80 °C prior to analysis.

The sweat samples were obtained in an air-conditioned room at 26 °C, but the ambient humidity was not controlled. Hands were washed with running water for 1 min and were wiped lightly with KimWipes® (Nippon Paper Crecia). The hands were then dried in air for 10 min without direct contact with anything to avoid contamination. Two milliliters of 10% ethanol was poured into an uncovered round dish (the inner diameter of 35 mm and the depth of 10 mm, Tissue Culture Dish®, Becton Dickson). The dish was covered with a patient's right palm, and was turned around to wet the palm with 10% ethanol while tightly holding the patient's palm on the dish. While tightly covering the patient palm with the dish, the hand was slowly rocked for 30 s to thoroughly collect all substances on skin surface. The hand was turned around again to collect the solution in the dish. The solutions were kept at −80 °C prior to analysis.

2.3. Estimation of the amount of palm sweating

To estimate the amount of sweat secreted from the palm, a hand-assembled perspiration meter (Pico-Device) was fitted distal to the PIP joint of the left index finger.

2.4. Sample preparations

The phenylboronate monolithic spin column (GL Sciences) was conditioned with acetic acid and phosphate buffer. First, 0.2 ml of 1% acetic acid was added to the column, and the column was centrifuged at 10,000 ×*g* for 1 min. Then, 0.2 ml of 100 mmol/l phosphate buffer (pH 8.0) was added to the column, and the column was centrifuged at 10,000 ×*g* for 1 min. The analyte was then applied to the conditioned monolithic spin column, and the column was centrifuged at 10,000 ×*g* for 1 min. The column was then rinsed with 200 μl of 100 mmol/l phosphate buffer (pH 8.0), and centrifuged. Analytes were eluted from the column with 1% acetic acid (50 μl), and 40 μl eluates were used for HPLC analysis.

2.5. L-Dopa analysis

The HPLC system consisted of a pump (PU1580, Jasco), a manual sample injector (Rheodyne 7725), and an electrochemical detector (ED703, GL Sciences). Separations were performed on an ODS column (Inertsil ODS-4 column, 250 × 3.0 mm I.D., 5 μm, GL Sciences) at 35 °C. The mobile phase was composed of 20 mmol/l sodium acetate–citrate buffer/acetonitrile (100/5, v/v) containing 1 g/l sodium 1-octanesulfonate at a flow-rate of 0.5 ml/min.

3. Results

3.1. Sweat sampling

In this study, we collected sweat from the palm. The sweating of both fingers and palms is generally affected by emotional and also thermoregulatory impulses. First we collected sweat from the index finger. However, because of the resting tremor of the patients, taking a sweat sample from the finger was more difficult than we predicted. Hence, we collected sweat from the palm. The amount of sweat excreted from the palm (y) was estimated from the sweat on the finger (x) with the following equation, $y = 0.50x$ ($R^2 = 0.77$, $n = 12$). The sweating rate of the palm was thus nearly half of that of the finger. The amount of L-dopa in the sweat sample was normalized by the amount of sweat from the index finger excreted during sample collection.

3.2. L-Dopa determination in sweat sample

L-Dopa concentrations in sweat samples were measured essentially according to our previously developed method [8]. This method was applicable to the determination of plasma L-dopa concentrations in PD patients taking the drug. However, L-dopa concentrations in sweat samples were much lower than those in plasma. Hence, we modified the pretreatment steps in the previous method. We used phenylboronate-modified monolithic silica spin columns because phenylboronate forms a stable, anionic complex with the *cis*-hydroxyl groups of catechol compounds [9]. With the monolithic silica spin column, we concentrated the sample by applying 200 μl sweat sample and eluting it with 50-μl elution buffer. With the 4-fold concentration of the sample, the final extraction recovery of L-dopa from the sweat sample was more than 80%.

Fig. 1A shows the chromatogram of a standard sample of L-dopa. L-Dopa was retained with a retention time of ~5 min. Lack of the L-dopa peak was confirmed in 3 controls (Fig. 1B). A representative chromatogram obtained from a sweat sample of a PD patient is shown in Fig. 1C. The peak of L-dopa was clearly identified without any interference from the other substances in the sweat sample. There might be some endogenous catechol compounds such as dopamine and norepinephrine, and they were not found on the chromatogram probably because their concentrations in sweat samples were not high.

The linear response of the electrochemical detector signal ranged from 5 nmol/l to 5 μmol/l. The precision and accuracy of the method are summarized in Table 1. The validation data indicates that the method was reliable for the quantitative analysis of L-dopa in sweat samples.

3.3. Relationship between plasma and sweat L-dopa concentrations

We explained our study design to 23 PD patients who were followed up by MH and all agreed to give signed informed consent to participate in the study. We did not ask severely disabled patients, who could not easily hold their hands extended and still, to join our study. Ages of the patients were 65.2 ± 8.0 y (mean ± SD, range 48 to 78 y), and the disease durations were 6.5 ± 3.8 y (range 1.5 to 13 y). Hoehn and Yahr scales were 2.19 ± 0.76 (range 1 to 4). UPDRS2 and UPDRS3 scores were 9.3 ± 4.5 (range 3 to 21) and 17.3 ± 7.8 (range 4 to 31),

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