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RESEARCH PAPER

Elevation of Plasma Levels of L-Dopa in Transdermal Administration of L-Dopa-Butylester in Rats

Jun-ichi Sudo,[1,*] Hiroaki Iwase,[2]
Kimio Higashiyama,[3] Katsuhiko Kakuno,[1]
Fumiji Miyasaka,[1] Takashi Meguro,[1] and
Kozo Takayama[2]

[1]*Department of Clinical Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan*
[2]*Department of Pharmaceutics and*
[3]*Institute of Medicinal Chemistry, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan*

ABSTRACT

To increase delivery of L-dopa in its transdermal absorption, a new lipophilic derivative of L-dopa, L-dopa-butylester, was synthesized. An in-vitro study employing two-chamber diffusion cells, in which the excised rat abdominal skin was mounted, revealed that, in the presence of L-menthol and ethanol, L-dopa-butylester penetrated in its original form more effectively than L-dopa. L-Dopa-butylester sheets were made by immersing wiper sheets in methanol containing the compound, and then evaporating the methanol. An extraction study of the compound from the sheets revealed that its stability was maintained for at least 12 weeks. In an in-vivo cutaneous absorption study, an L-dopa-butylester sheet was attached to the shaved rat abdominal skin. A hydrogel containing L-menthol and ethanol was spread on vinyl tape, and this sheet was placed over it. In plasma, the L-dopa level rose linearly between 30 and 180 min after the cutaneous application; L-dopa-butylester was not detected. The L-dopa level was

*Corresponding author. 2-Jo, 2-Chome, 6-5, Ainosato, Kita-ku, Sapporo, Hokkaido 002-8072, Japan. Fax: +81-11-778-7103; E-mail: j-sudo@phoenix-c.or.jp

higher than that in which L-dopa was applied. These findings indicated that the lipophilic nature of L-dopa-butylester further increased its penetration through the skin, and that L-dopa-butylester that was taken up into the general circulation system was rapidly converted to L-dopa by hydrolysis in the body.
Key Words: *L-Dopa; L-Dopa-butylester; Hydrogel; Transdermal absorption; Rat*

INTRODUCTION

L-Dopa is a therapeutic agent used for Parkinson's disease (1,2). In Parkinson's disease patients, complications of dysphagia and dementia frequently occur. When such patients attempt to swallow drugs, they encounter difficulties and may choke, causing dysphagial pneumonia.

To cope with this problem, we previously developed a transdermal absorption system with a hydrogel containing L-dopa (3). Furthermore, to maintain the stability of L-dopa, we developed another system composed of two separate layers of L-dopa and hydrogel (4). However, the systems were estimated to give too low plasma level of L-dopa for patients with a severe condition (5).

Thus, to increase plasma levels of L-dopa, we synthesized a lipophilic derivative of L-dopa, L-dopa-butylester, and examined the effectiveness its transdermal absorption in vitro and in vivo in rats.

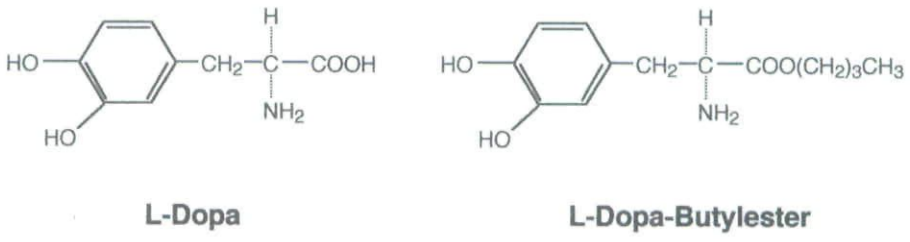
MATERIALS AND METHODS

Synthesis of L-Dopa-butylester

L-Dopa-butylester (butyl 3,4-dihydroxyphenyl-alaninate) (Fig. 1) was synthesized by the method of Vaughan and Joyce (6), A 7.9 g amount of L-dopa (L-3-4-dihydroxyphenylalanine; Sigma Chemicals, St Louis, MO) was suspended in 120 ml of absolute 1-butanol. The suspension was saturated for 1 hr with hydrogen chloride, and heated for an additional 1 hr under reflux. The solvent in the suspension was evaporated in vacuo. The oil residue was dissolved in 300 ml of water, and alkalized with ammonium hydroxide solution. Colorless crystals that began to appear in the solution on standing were collected by filtration. Recrystallization was performed with ethyl acetate. The purity of this compound was characterized by nuclear magnetic resonance (NMR) spectroscopy, and was greater than 99%.

In Vitro Cutaneous Permeation Study

Cutaneous permeation of L-dopa-butylester was investigated in the same way as previously reported (3,7). Full-thickness abdominal skin was excised from male Wistar strain rats (weighing 300 ± 10 g, Saitama Laboratory Animals, Saitama, Japan), whose hair had been removed beforehand by an electric clipper. The skin excised was immediately used as a permeation membrane. Two-chamber diffusion cells (available diffusion area, 0.785 cm^2; volume of each half-cell, 3.0 ml) with a water jacket were employed. L-Dopa-butylester was suspended with excess amounts (6.42 g/dl: 5.00 g/dl as L-dopa equivalent) in the following solutions: water; 20% (v/v) ethanol and 2% (w/v) L-menthol solution; and



L-Dopa **L-Dopa-Butylester**

Figure 1. Chemical structures of L-dopa and L-dopa-butylester. Molecular weights: L-dopa = 197.19; L-dopa-butylester = 253.29.

40% ethanol and 2% L-menthol solution. The suspension in each of the three solutions was applied to the donor cell, and the receiver cell was filled with phosphate-buffered saline (pH 7.4). Both cells were warmed at 40°C and stirred by magnetic stirrers. At 0 (before), 30, 60, 120, and 180 min after the L-dopa-butylester suspension was applied to the donor cell, 1 ml aliquots were withdrawn from the receiver cell. Thereafter, an equivalent volume of the phosphate-buffered saline was supplied to the receiver cell.

For comparison with the data of L-dopa-butylester, L-dopa in an excess amount (5.00 g/dl) was suspended in 40% ethanol and 2% L-menthol solution in the donor cell, and treated in a similar manner.

The aliquots collected were diluted with 0.2 M perchloric acid for L-dopa determination, or with methanol for L-dopa-butylester determination. After centrifugation (10,000 g, 10 min, 4°C), the supernatants were analyzed by high performance liquid chromatography (HPLC), as described below.

Preparation of L-Dopa, L-Dopa-butylester-, and Hydrogel Sheets

Wiper sheets (type CR-4; Nippon Kimberly-Clark, Tokyo, Japan) were cut in a rectangular shape (1 cm × 3 cm).

L-Dopa was dissolved in 5 M hydrochloride at a concentration of 100 mg/ml. The wiper sheet was immersed in 2 ml of the L-dopa solution, and then lyophilized (L-dopa sheet).

L-Dopa-butylester was dissolved in methanol at a concentration of 100 mg/ml. The wiper sheet was immersed in 1 ml of the L-dopa-butylester solution, and then evaporated in vacuo (L-dopa-butylester sheet).

The hydrogel was prepared as previously reported (4), and 1 g of the hydrogel was spread on a vinyl tape (Clear Tape, type CK-24; Sumitomo-3M, Tokyo, Japan) (hydrogel sheet).

Stability Examination of L-Dopa and L-Dopa-butylester Sheets

The L-dopa and L-dopa-butylester sheets were kept at room temperature in a dark box for 0, 2, 6, and 12 weeks after the preparation. On the given day, the L-dopa sheet was put into 100 ml of 0.2 M perchloric acid solution, and the L-dopa-butylester sheet was also put into 100 ml of methanol. After stirring for 1 h using a magnetic stirrer, the aliquots were diluted with the extraction fluid. After centrifugation (10,000 *g*, 10 min, 4°C), the supernatants were applied to the HPLC, as described below.

In Vivo Cutaneous Absorption Study

Male Wistar strain rats (weighing 300 ± 10 g) were anesthetized with ether, and the left jugular vein was catheterized with a polyethylene tube (PE-50). Through this route, saline was continuously infused at 0.1 ml/kg body weight/min, and urethane (500 mg/kg body weight) and α-chloralose (70 mg/kg body weight) were administered for deeper anesthesia and immobilization (3). The animal was intubated for free respiration. Then, the abdominal hair was gently removed using electric clippers. Either an L-dopa sheet or an L-dopa butylester sheet was attached to the shaved abdominal skin, and a hydrogel sheet was placed over it. Thirty minutes before collection of blood, the left femoral artery was catheterized with a polyethylene tube (PE-50) that had been filled with 0.2 M *O*,*O*′-bis(2-aminoethyl)ethyleneglycol-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) dissolved in saline (8). Blood was collected at 0 (before), 30, 60 and 180 min after applying the two layers of L-dopa (or L-dopa-butylester) and hydrogel, and then put into chilled tubes containing 40 µl of a solution containing 0.2 M EGTA and 0.2 M reduced glutathione (9).

Control rats were operated on in the same manner, but received neither L-dopa nor L-dopa-butylester.

Blood samples were centrifuged (1700 *g*, 10 min, 4°C) to obtain plasma.

For determination of L-dopa and catecholamines, the plasma were pretreated by the method of Eriksson and Persson (9), and applied to the HPLC, as described below.

For determination of L-dopa-butylester, 9 volumes of methanol was added to 1 volume of the plasma, and mixed. After centrifugation (10,000 *g*, 10 min, 4°C), the supernatant was further filtered using an 0.2 µm-filter. The filtrate was applied to the HPLC, as described below.

Determination of L-Dopa-butylester, L-Dopa, and Catecholamines

L-Dopa-butylester was electrochemically determined by the following HPLC method: column, Mightysil RP-18 (5 μm, 4 × 250 mm, Kanto Chemicals, Tokyo, Japan); mobile phase, 12% acetonitrile in 10 mM sodium dihydrogenphosphate/0.01 mM EDTA (pH 3.1); flow rate, 1.0 ml/min; column temperature, 40°C; detection, electrochemically at +0.7 V; in this HPLC system, the retention time of L-dopa-butylester was 22.2 min.

L-dopa, dopamine, norepinephrine, and epinephrine in the aliquots were electrochemically determined using the HPLC method, as reported previously (3).

Statistics

Values were expressed as means ± SEM. The data were subjected to analysis of variance, and significant differences were defined among groups by Bonferroni's method; p values less than 0.05 were considered significant.

RESULTS

In Vitro Cutaneous Permeation Study

Cumulative amounts of L-dopa-butylester that permeated through the skin into the receiver cell were determined (Fig. 2a). In addition, cumulative amounts of L-dopa that were derived from L-dopa-butylester were also determined in the receiver cell (Fig. 2b).

L-Dopa-butylester suspension in water revealed that L-dopa-butylester had cutaneously penetrated through the skin into the receiver cell with the time elapsed: at 180 min, 31.65 ± 8.03 g/cm^2. Cumulative amounts of L-dopa in the receiver cell were also increased within the time elapsed: at 180 min, 1.13 ± 0.08 μg/cm^2.

The results obtained with the L-dopa-butylester suspension in 20% ethanol and 2% L-menthol solution indicated that the accumulations of L-dopa-butylester and L-dopa were increased in the receiver cell, and were further increased with an increase in the ethanol concentration to 40%. The L-dopa-butylester suspension in 40% ethanol and 2% L-menthol solution results suggested that, at 180 min, 95% of the permeated L-dopa-butylester was in the original form, and the residual 5% was in the converted form, L-dopa.

The cumulative amounts of L-dopa-butylester and L-dopa were calculated as L-dopa equivalents (Fig. 2c). These findings indicated that when the ethanol was increased to 40%, the L-dopa-butylester suspended in the donor cell permeated the skin to the greatest extent. When either

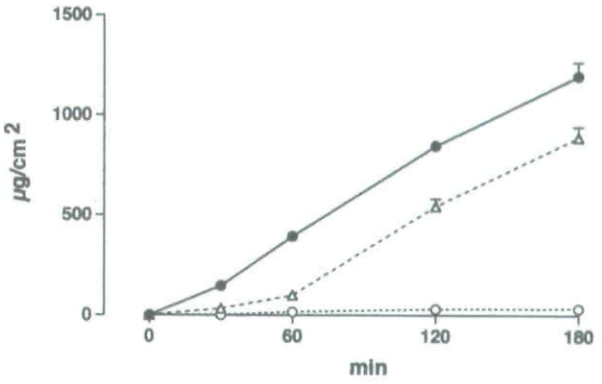


Figure 2a. Cumulative amount of L-dopa-butylester observed through rat abdominal skin in an in-vitro study employing two-chamber diffusion cells. L-Dopa-butylester was suspended in water (○), 20% ethanol and 2% L-menthol (△), and 40% ethanol and 2% L-menthol (●) in the donor cell. Points and bars: means ± SEM in five experiments.

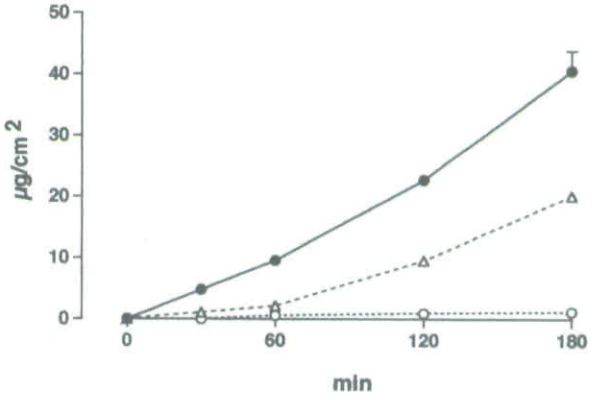


Figure 2b. Cumulative amount of L-dopa observed through rat abdominal skin in an in-vitro study employing two-chamber diffusion cells. L-Dopa-butylester was suspended in water (○), 20% ethanol and 2% L-menthol (△), and 40% ethanol and 2% L-menthol (●) in the donor cell. Points and bars: means ± SEM in five experiments.

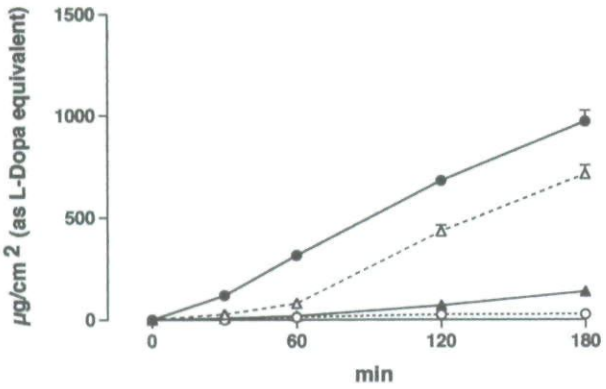


Figure 2c. Cumulative amount of sum of L-dopa-butylester and L-dopa observed through rat abdominal skin in an in-vitro study employing two-chamber diffusion cells. L-Dopa-butylester was suspended in water (○), 20% ethanol and 2% L-menthol (△), and 40% ethanol and 2% L-menthol (●) in the donor cell. L-dopa was suspended in 40% ethanol and 2% L-menthol in the donor cell (▲). Points and bars: means ± SEM in five experiments.

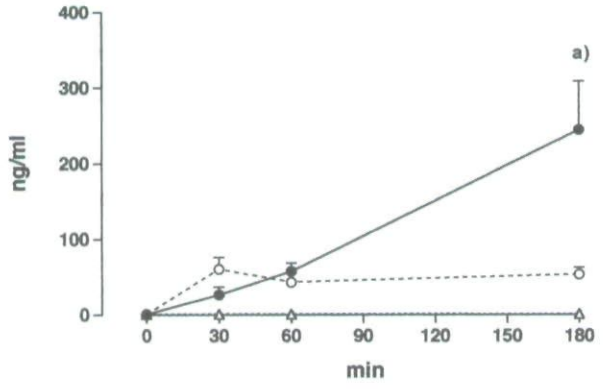


Figure 3. Plasma level of L-dopa during transdermal administration of L-dopa and L-dopa-butylester. Either an L-dopa-butylester sheet or an L-dopa sheet was attached to the shaved rat abdominal skin. A hydrogel sheet containing 40% ethanol and 2% L-menthol was placed over it. (△), Control; (○), L-dopa; (●), L-dopa-butylester. Points and bars: means ± SEM. Seven rats in each group. Statistics: a), $p < 0.05$, compared with the L-dopa administration.

L-dopa or L-dopa-butylester was suspended in the 40% ethanol and 2% L-menthol solution, the amounts permeated at 180 min were approximately 7-fold larger with L-dopa-butylester than with L-dopa.

Stability Examination of L-Dopa and L-Dopa-Butylester Sheets

Hydrogels that contained L-dopa or L-dopa-butylester showed blackish coloration several days after their preparations. Thus, another system composed of two separate layers of the agents and hydrogel (4) was employed in the studies below, and stability of the agents in the sheets was examined.

On the day (day 0) the sheets were prepared, the amount of L-dopa that was extruded from the L-dopa sheet was 185.1 ± 3.4 mg/sheet ($n = 7$), and that of L-dopa-butylester extracted from the L-dopa-butylester sheet was 72.1 ± 6.2 mg/sheet ($n = 7$). These values remained unchanged until 12 weeks: in the L-dopa sheets the amount at 12 weeks was 95.3% of that on day 0; in the L-dopa-butylester sheets the amount at 12 weeks was 96.2% of that on day 0. The colors of the L-dopa and L-dopa-butylester sheets were white on day 0, and they remained so for 12 weeks.

In Vivo Cutaneous Absorption Study

Either an L-dopa-butylester sheet or an L-dopa sheet was attached to the shaved rat abdominal skin. A hydrogel containing 40% ethanol and 2% L-menthol was spread on vinyl tape, and this sheet was placed over it.

Administration of L-dopa-butylester revealed that no L-dopa-butylester was detected in the plasma throughout the entire experimental period; its detection limit in the system employed was 0.8 ng/ml.

The L-dopa levels of the control rats were around 0.7 ng/ml throughout the entire experimental period. After L-dopa administration the L-dopa level peaked at 30 min, and reached a plateau (approximately 50 ng/ml) between 60 and 180 min (Fig. 3). In contrast, the L-dopa-butylester administration revealed that the L-dopa level was elevated linearly during 30 and 180 min; at 180 min, the level was approximately 5-fold higher than that after L-dopa administration (Fig. 3).

The dopamine levels of the control rats were around 0.3 ng/ml throughout the entire experimental period. The L-dopa administration showed a higher level only at 180 min compared with that of the control (Fig. 4). The L-dopa-butylester administration elevated the dopamine level from 30 min,

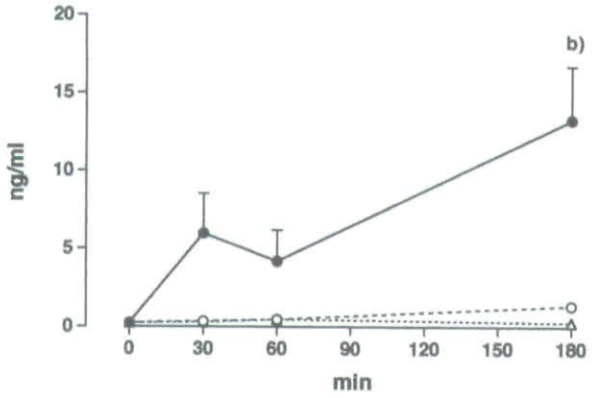


Figure 4. Plasma level of dopamine during transdermal administration of L-dopa and L-dopa-butylester. Either an L-dopa-butylester sheet or an L-dopa sheet was attached to the shaved rat abdominal skin. A hydrogel sheet containing 40% ethanol and 2% L-menthol was placed over it. (△), Control; (○), L-dopa; (●), L-dopa-butylester. Points and bars: means ± SEM. Seven rats in each group. Statistics: b), $p < 0.01$, compared with the L-dopa administration.

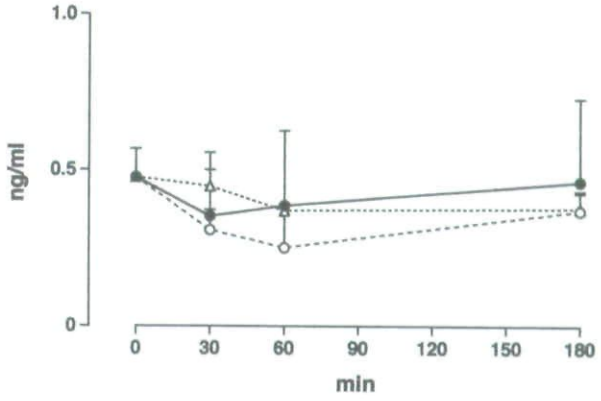


Figure 5. Plasma level of norepinephrine during transdermal administration of L-dopa and L-dopa-butylester. Either an L-dopa-butylester sheet or an L-dopa sheet was attached to the shaved rat abdominal skin. A hydrogel sheet containing 40% ethanol and 2% L-menthol was placed over it. (△), Control; (○), L-dopa; (●), L-dopa-butylester. Points and bars: means ± SEM. Seven rats in each group. There was no significant difference among the groups.

showing its maximum at 180 min; the level at 180 min was approximately 10-fold higher than that after L-dopa administration (Fig. 4).

The administrations of L-dopa and L-dopa-butylester showed no significant change in the norepinephrine level (Fig. 5).

The epinephrine levels of the control rats remained at approximately 0.6 ng/ml throughout the entire experimental period. After L-dopa administration the epinephrine level reached a peak at 60 min and slightly declined at 180 min (Fig. 6). After L-dopa-butylester administration the level of epinephrine also reached a peak at 60 min, which was maintained for an additional 120 min (Fig. 6).

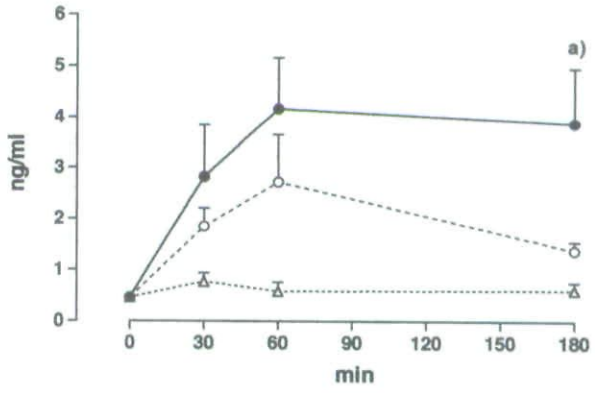


Figure 6. Plasma level of epinephrine during transdermal administration of L-dopa and L-dopa-butylester. Either an L-dopa-butylester sheet or an L-dopa sheet was attached to the shaved rat abdominal skin. A hydrogel sheet containing 40% ethanol and 2% L-menthol was placed over it. (△), Control; (○), L-dopa; (●), L-dopa-butylester. Points and bars: means ± SEM. Seven rats in each group. Statistics: a), $p < 0.05$, compared with the L-dopa administration.

DISCUSSION

First, we employed two-chamber diffusion cells using the excised rat abdominal skin as a permeation membrane, and investigated the effectiveness of L-menthol and ethanol as absorption enhancers on the transdermal absorption of L-dopa-butylester. This in-vitro study confirmed that ethanol and L-menthol effectively changed the dense barrier structure of the stratum corneum (7), and that, as a result, the diffusivity of L-dopa-butylester through the skin had been increased. In addition, the present findings also indicated that the applied L-dopa-butylester mostly penetrated through the skin in its original form.

L-Dopa-butylester in the hydrogel that contained ethanol and L-menthol resulted in blackish coloration within several days after its preparation. Thus, using a system composed of two separate layers of L-dopa-butylester and hydrogel (4), we performed the stability examination and the in-vivo study.

The stability examination of L-dopa and L-dopa-butylester from their respective sheets indicated that the two agents in the sheets were stable for at least 12 weeks, provided the sheets were kept at room temperature in a dark box.

In the present study, the final amount of L-dopa that remained in the sheet was 185.1 mg. In a previous study, a sheet that contained 12.98 mg of L-dopa was prepared, and the same animal experiment was performed (4). Although the L-dopa content in the present study was approximately 14-fold greater than in the previous study, the extents of elevation of plasma L-dopa were similar in the studies (4). This finding suggested that the elevation was near maximum, even when more than 12.98 mg of L-dopa/sheet was applied to the rat skin.

In terms of the L-dopa-butylester sheet, the final amount of L-dopa-butylester in the sheet was 72.1 mg/sheet: this corresponded to 57.9 mg/sheet as the L-dopa equivalent. Accordingly, when estimated as the L-dopa equivalent, the amount of L-dopa-butylester/sheet was approximately one-third of that of L-dopa/sheet employed in the present study. Nevertheless, the present in-vivo cutaneous absorption study indicated that the application of L-dopa-butylester more effectively elevated the plasma level of L-dopa than the application of L-dopa. Thus, the lipophilic nature of L-dopa-butylester further increased its penetration through the dense barrier structure that was loosened by ethanol and L-menthol. In addition, L-dopa-butylester passed through the skin in its original form in the in-vitro study, and L-dopa-butylester was not detected in the plasma in the in-vivo study. These findings in vitro and in vivo suggest that the L-dopa-butylester, which passed through the skin, was hydrolyzed in other organs than the skin, such as the plasma and liver.

Furthermore, our in-vivo study indicated that the plasma level of L-dopa maintained as a plateau during 60–180 min after the L-dopa-administration, while the level was linearly elevated until 180 min by the L-dopa-butylester administration. We considered that, to confirm whether L-dopa-butylester administration resulted in the steady-state plasma level of L-dopa, the experimental period should be more prolonged.

In addition, the present application of L-dopa-butylester with the hydrogel revealed that dopamine and epinephrine levels rose throughout the entire experimental period. Although the elevated levels of dopamine and epinephrine were considered to have resulted from enzymatic conversions in the body (10–12), such elevations in plasma catecholamines might exert digestive, circulatory, psychiatric, and other effects (2,13). Thus, further improvement is required to minimize these adverse effects (14) before clinically applying this transdermal delivery system of L-dopa-butylester to humans.

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