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COMMUNICATION

Transdermal Absorption of L-Dopa from a New System Composed of Two Separate Layers of L-Dopa and Hydrogel in Rats

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ABSTRACT

To maintain the stability of L-dopa in hydrogel, a new system composed of two separate layers of L-dopa and hydrogel was developed. L-Dopa sheets were made by immersing L-dopa solution into wiper sheets and by lyophilizing them. Examination for stability of L-dopa in the L-dopa sheet revealed that its stability was maintained for at least 12 weeks, providing the sheet was kept at room temperature in a dark box. In a cutaneous absorption study of L-dopa in rats, an L-dopa sheet was attached to the shaved abdominal skin. A hydrogel composed of cutaneous absorption enhancers, water and ethanol, was spread on vinyl tape (hydrogel sheet), and this sheet was placed over the L-dopa sheet. L-Dopa that was administered transdermally effectively penetrated through the skin: The plasma level of L-dopa peaked at 30 min and remained high between 60 and 180 min after the cutaneous application. Our system, composed of two separated layers of L-dopa and hydrogel, enabled the stability of L-dopa to be maintained without losing transdermal absorption of L-dopa.

Key Words: *L-Dopa; Hydrogel; Rat; Transdermal absorption.*

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INTRODUCTION

L-Dopa is a therapeutic agent used for treatment of Parkinson's disease (1,2). In patients with Parkinson's disease, complications of dysphagia and dementia occur frequently. When such patients attempt to swallow drugs, they encounter difficulty and may choke, causing dysphagial pneumonia. In this case, drug administration by injection is preferable. However, cooperation cannot be obtained from patients with dementia, making injection quite difficult; the patient might disengage the intravenous drip injection needle from the arm, resulting in excessive bleeding.

To cope with this problem, we previously developed a transdermal absorption system with a hydrogel containing L-dopa (3). In animal experiments using rats, L-dopa from the hydrogel effectively penetrated into the body through the skin; however, the L-dopa hydrogel became blackish within several days after its preparation (3). Since the color of the hydrogel not containing L-dopa did not change, the blackish coloration was attributed to the instability of L-dopa in the hydrogel. To maintain the stability of L-dopa, we developed a system composed of two separate layers of L-dopa and hydrogel and examined the stability of L-dopa and the effectiveness of transdermal absorption of L-dopa in rats.

MATERIALS AND METHODS

Animals

Male Wistar strain rats (Saitama Laboratory Animals Co., Saitama, Japan) weighing 300 ± 10 g were housed in ordinary cages and allowed free access to water and a standard pellet diet (CE-2; Clea Japan Co., Tokyo, Japan) prior to the study.

Preparation of L-Dopa and Hydrogel Sheets

L-Dopa (L-3-4-dihydroxyphenylalanine; Sigma Chemicals Co., St Louis, MO) was dissolved in 5 M hydrochloride in a concentration of 25 mg/ml. A wiper sheet (type CR-4, Nippon Kimberly-Clark Co., Tokyo, Japan) was cut in a rectangular shape (1 cm × 3 cm). The L-dopa solution (1 ml) was immersed into the sheet and lyophilized (L-dopa sheet).

A hydrogel was prepared with the following composition (100 g total): 10 g propylene glycol, 2 g L-menthol, 2 g diisopropyl adipate, 1 g diisopropanolamine, 1 g carboxyvinyl polymer, 40 g ethanol, and 44 g water. Vinyl tape (Clear Tape, type CK-24, Sumitomo-3M Co., Tokyo, Japan) was spread with 1 g of the hydrogel (hydrogel sheet).

Extraction of L-Dopa from the L-Dopa Sheet

The L-dopa sheets were kept at room temperature in a dark box for 0, 2, 6, and 12 weeks after preparation. On the given day, each of the L-dopa sheets was put into 100 ml of 0.1 M perchloric acid solution, followed by stirring for 1 hr using a magnetic stirrer. The amount of L-dopa in the sheet was calculated by determining the L-dopa concentration in the solution.

Cutaneous Absorption Study

Rats were anesthetized with ether, and the left jugular veins were 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 given for deeper anesthesia and immobilization (3). The animal was intubated for free respiration. Then, the abdominal hair was gently removed using electric clippers. An L-dopa sheet was attached to the shaved abdominal skin, and a hydrogel sheet was placed over it (Fig. 1). The left femoral artery was catheterized 30 min before collection of blood with a polyethylene tube (PE-50) that had been filled with 0.2 M EGTA dissolved in saline (4). Blood was collected at 0 (before), 30, 60, and 180 min after applying the two layers of L-dopa 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 (5).

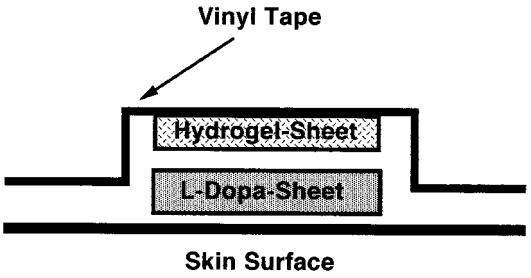

Figure 1. Illustration of cutaneous attachment of L-dopa and hydrogel. A sheet that contained L-dopa was attached to shaved abdominal skin of rats, and a hydrogel sheet was placed over it.

Control rats were operated on in the same manner, but did not receive L-dopa.

Determination of L-Dopa

Blood samples were centrifuged (1700*g*, 10 min, 4°C) to obtain plasma. Aliquots were pretreated by the method of Eriksson and Persson (5). L-Dopa, dopamine, norepinephrine, and epinephrine in the samples were determined electrochemically by high-performance liquid chromatography as previously reported (4).

Statistics

Data were expressed as means ± SEM and were analyzed statistically using the Student unpaired *t* test; *P* values less than .05 were considered significant.

RESULTS

Extraction of L-Dopa from L-Dopa Sheets

On the day (day 0) the L-dopa sheets were prepared, the amount of L-dopa that was extruded from the L-dopa sheet was 12.98 ± 0.46 mg per sheet ($N = 7$) (Fig. 2). This value remained unchanged until 12 weeks: The amount at 12 weeks was 95.3% of that on day 0. The color of the L-dopa sheet was white on day 0, and it remained so for 12 weeks.

Cutaneous Absorption of L-Dopa

L-Dopa and the amines in the plasma were determined after the L-dopa and the hydrogel sheets were attached to the rat abdominal skin. The levels of L-dopa and dopamine reached a peak at 30 min, decreased at 60 min, and again rose at 180 min (Figs. 3 and 4). The norpinephrine level showed no significant change (Fig. 5). The epinephrine level reached a peak at 60 min, but decreased at 180 min (Fig. 6).

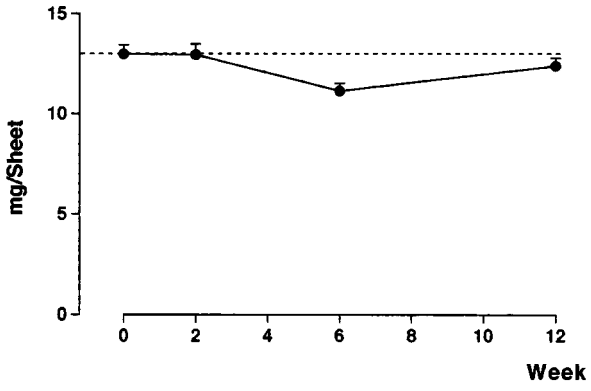

Figure 2. Amount of L-dopa extruded from L-dopa-sheets. Points and bars: means ± SEM of seven experiments at each time point.

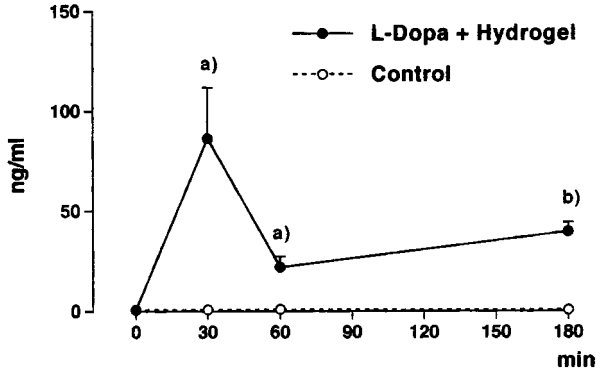

Figure 3. Plasma level of L-dopa during cutaneous attachment of L-dopa and hydrogel. Points and bars: mean ± SEM; seven rats in each group: (a) $P < .05$; (b) $P < .01$, compared with the control at identical time points.

DISCUSSION

For preparation of L-dopa sheets, 25 mg of L-dopa was immersed into a wiper sheet. However, the final amount of L-dopa that resided in the sheet after completion of the immersion and lyophilization was 12.98 mg.

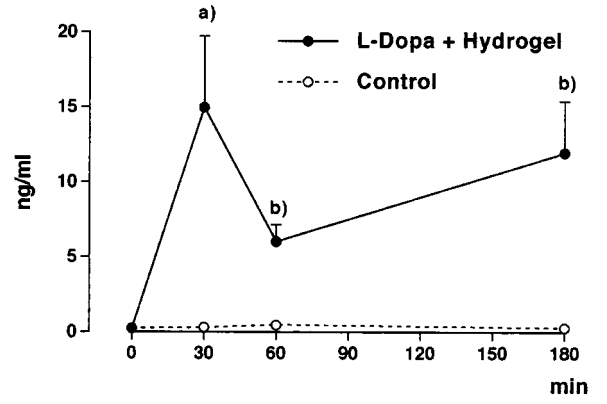

Figure 4. Plasma level of dopamine during cutaneous attachment of L-dopa and hydrogel. Points and bars: mean ± SEM; seven rats in each group: (a) $P < .05$; (b) $P < .01$, compared with the control at identical time points.

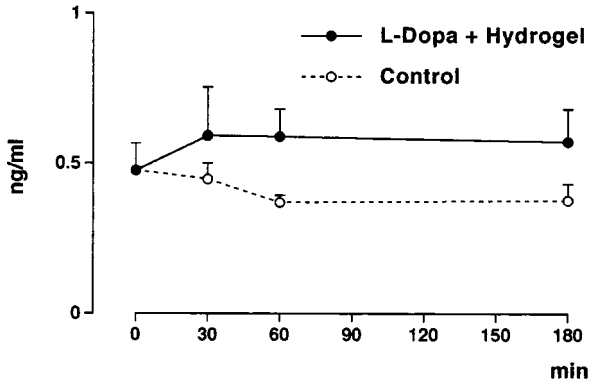

Figure 5. Plasma level of norepinephrine during cutaneous attachment of L-dopa and hydrogel. Points and bars: mean ± SEM. Seven rats in each group.

Examination of the stability of L-dopa in the L-dopa sheets revealed that the amount of L-dopa in the sheets was unchanged until 12 weeks, and that the color of the sheets remained white throughout the entire observation period. These findings indicated that the L-dopa in the sheets was stable for at least 12 weeks, providing the sheets are kept at room temperature in a dark box.

This study revealed that our system, composed of separate layers of L-dopa and hydrogel, effectively allowed L-dopa to penetrate through the skin. This finding suggests that the combination of 40% ethanol and 2% L-menthol altered the dense barrier structure of the stratum corneum (6,7), resulting in increased diffusion of L-dopa through the skin (3).

Application of the L-dopa and the hydrogel sheets on the rat skin revealed that the plasma level of L-dopa rose during 30 and 180 min, showing its peak at 30 min. These changes in the plasma levels of L-dopa could be explained by the changes in the concentrations of L-dopa in the hydrogel that was attached to the skin surface: (*a*) the concentration of L-dopa in the hydrogel on the skin surface was the highest immediately after application of the L-dopa and the hydrogel sheets on the skin; (*b*) then, the concentration gradually decreased by diffusion of L-dopa into the hydrogel; and (*c*) the concentration finally became equilibrated by diffusion and attained a constant level.

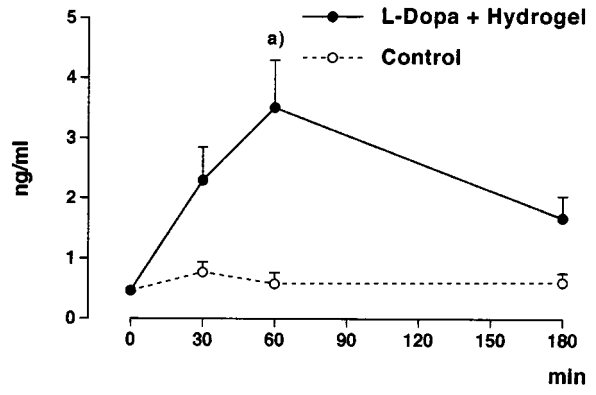

Figure 6. Plasma level of epinephrine during cutaneous attachment of L-dopa and hydrogel. Points and bars: mean ± SEM; seven rats in each group: (a) $P < .05$.

The maintenance of a constant plasma level of L-dopa was considered to be advantageous for patients who exhibit the ''wearing-off'' phenomenon since this phenomenon was partially attributed to alteration of the plasma concentration of L-dopa (8). Although the present study revealed that the plasma L-dopa remained within a limited range between 60 and 180 min, the rapid elevation of plasma L-dopa in the first 30 min was considered to be disadvantageous for the patients exhibiting the wearing-off phenomenon. Therefore, further improvement in our system is needed to maintain a relatively constant level of plasma L-dopa.

In addition, our application of the L-dopa and the hydrogel sheets on the rat skin showed that dopamine and norepinephrine levels rose throughout the entire experimental period. Aromatic L-amino acid decarboxylase (9), dopamine β-hydroxylase (10), and phenylethanolamine-*N*-methyltransferase (11) were found to be distributed in some organs of the body. Therefore, we considered that the enzymes in organs, as well as in the plasma, are involved in the decarboxylation of L-dopa, β-hydroxylation of dopamine, and *N*-methyltransformation of norepinephrine since L-dopa, dopamine, and norepinephrine remain in the body long enough for the enzymatic reactions in the organs.

Elevated levels of catecholamines in plasma exert digestive, circulatory, psychiatric, and other effects (2,12). Thus, the continuous elevation in the plasma levels of dopamine and epinephrine during the transdermal administration of the L-dopa hydrogel might induce the above adverse effects. Therefore, further improvement is required to minimize these adverse effects (13) to apply this transdermal delivery system of L-dopa to humans clinically. Nevertheless, our system with two layers of L-dopa and hydrogel allows L-dopa to remain stable for a long period. In addition, this system, composed of separate layers of L-dopa and hydrogel attached to the skin, does not cause pain. Thus, compliance in the administration of L-dopa to patients with Parkinson's disease with dysphagia would be improved.

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