Prolongation of Antidiuretic Response to Desmopressin Acetate by Iontophoretic Transdermal Delivery in Rats

Masashi Nakakura,* Yasuki Kato, and Kunio Ito

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagazumi-cho, Sunto-gun, Shizuoka 411, Japan. Received November 28, 1996; accepted February 1, 1997

An iontophoretic drug delivery system was compared with intranasal, oral and subcutaneous delivery from a standpoint of the prolongation of the antidiuretic response to desmopressin acetate (DDAVP) in diabetes insipidus rats. Iontophoretic delivery was comparable to the nasal route at a dose about five times higher than the nasal route dose, and was 2 to 3 times as effective as the oral route. Effect of dose and duration of current application on the prolongation of the response to DDAVP was also investigated in order to find the effectiveness of the iontophoresis. The antidiuretic response to DDAVP delivered by iontophoresis indicated a dose-dependent prolongation and was prolonged up to about 14 h with the increase of the duration of current application; when a pulsed direct current at 0.1 mA was passed for about 1 h, the response to DDAVP was prolonged for about 9 h. DDAVP in the anodic donor steeply decreased with the application for 1 h, and then gradually decreased. We suggest that the antidiuretic response to DDAVP can be effectively controlled by regulating the absorption of DDAVP at the short-term iontophoresis rather than prolonged treatment.

Key words iontophoresis; desmopressin acetate; skin; permeability; rat

Desmopressin acetate (DDAVP) is a derivative of vasopressin, and has an antidiuretic response. It is used in the treatment of neurogenic diabetes insipidus and nocturia.1,2) Many investigators have demonstrated the effectiveness of treatment of these diseases by oral and intranasal routes.3–6) However, these routes depend on passive diffusion and produce large inter-subject variabilities, so it is important that other routes of delivery is explored. Iontophoresis, using pulsed external electrical energy, has recently received attention as a promising technique to enhance the delivery of drug molecules across the skin.7–14) This technique could be of particular benefit for reducing the intersubject variabilities and controlling drug absorption.15,16) Ionized compounds and lower molecules are good candidates for iontophoretic delivery.11,17) Since DDAVP is a small peptide and exists as ions at physiological pH, transdermal iontophoretic delivery may be one possible route for the administration of DDAVP. DDAVP has been evaluated in some studies on transdermal delivery. For example, Kahn et al. reported transdermal delivery of DDAVP using the prodrug approach.18) Morimoto et al. reported that they enhanced the transdermal absorption of DDAVP by the combined application of iontophoresis and proteolytic enzyme inhibitors.12) We previously reported the effect of pulsatile iontophoresis on the DDAVP delivery.19) These reports suggest that DDAVP can be transdermally absorbed. On the other hand, it is necessary to maintain the antidiuretic response to DDAVP. However, few investigators have mentioned the prolonged response induced by a transdermal delivery of DDAVP. The goal of the present study was to assess the value of iontophoretic delivery of DDAVP from a standpoint of the prolongation of the antidiuretic response. Rats with diabetes insipidus were used as a model, and their antidiuretic response was monitored by measuring urine flow.20,21)

MATERIALS AND METHODS

Materials  DDAVP was supplied by Ferring AB (Malmo, Sweden). Isotonic sodium chloride solution and 5% glucose injection were obtained from Otsuka Pharmaceutical Co. (Tokyo, Japan). Urethane and citric acid were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Junsei Chemical Co. (Tokyo), respectively. All other reagents were obtained from Kanto Chemical Co. (Tokyo).

In Vivo Study  Urethane was intraperitoneally administered at 1 g/kg for anesthesia in male Wistar rats (280—350 g). The animal was fixed to a thermosatically regulated plate at 35 to 37°C. The urinary tract (penis) was closed with a string and the urinary bladder was exposed with an about 1 cm incision through the abdomen. The bladder was cannulated with a polyethylene tube (PE50, Clay Adams, Becton Dickinson and Company, New Jersey, U.S.A.) to collect urine specimens. Before each iontophoresis experiment, a hypotonic solution consisting of a mixture of (27:36:37) of isotonic sodium chloride solution, 5% glucose injection and water for injection (175 ± 5 mOsm/kg) was injected continuously for 16—20 h via a polyethylene cannula in the femoral vein to maintain the urine volume at about 1 ml. The solution was injected at a constant rate of 3 ml/h with a syringe pump (STC-523, 525, Terumo, Tokyo) in all experiments. The rat urine was collected every 15 min and the urine volume was checked as a pharmaceutical response to DDAVP. Each experiment was carried out with three or four rats.

Administration of Drug  Adhesive two cylindrical-type iontophoretic applicators (made of silicone, 10 mm i.d., 30 mm height) were fixed 10 mm apart on the cleanly shaven abdominal surface using an electric clipper. DDAVP solution (0.1, 1, 10 μg DDAVP in 1 ml of pH 6.0 citric acid–sodium hydroxide buffer solution) and 1 ml of isotonic sodium chloride solution were placed in the drug reservoir (anode applicator) and the reference reservoir (cathode applicator), respectively. Platinum electrodes, 8 mm in diameter, were immersed in each reservoir at
about 5 mm from the skin surface. Iontophoresis was conducted with an electric stimulating apparatus (SEN-3301, Nihon Koden, Tokyo). A pulsed direct current of 0.1 mA (2000 Hz, duty 50%) was delivered to the platinum electrodes for 12 h in each experiment. When the effect of the duration of current application was investigated, the duration was varied: 1, 2, 3, 4, 6 and 12 h. The drug solution in the anodic applicator was taken at the end of current application and analyzed for DDAVP by HPLC. DDAVP solution in a volume 0.5, 0.025 and 0.5 ml was administered subcutaneously, intranasally and orally, respectively, under the same experimental conditions. The other conditions for this study are summarized in Table 1.

**Data Treatment** The mean duration of urine volume remaining under the critical level, which was calculated as the mean of four urine fractions collected before the administration of DDAVP, was judged as the prolongation of the antidiuretic response to DDAVP.

**HPLC Analysis** DDAVP was analyzed by HPLC using a Superspher 100 RP-18 (Kanto Chemical Co., Tokyo), 125 x 4.0 mm, 4 μm. The mobile phase consisted of a mixture (75:13:12) of 1/15 m phosphate buffer (pH 5.2) solution, acetonitrile and methanol containing 0.8 mg/ml sodium n-butansulfonate. Detection was performed by UV-detection at 220 nm wavelength.

**RESULTS AND DISCUSSION**

Comparison of Antidiuretic Response to DDAVP by Iontophoretic Administration with Those by Subcutaneous, Intranasal and Oral Administration A dose of 0.02 μg of DDAVP per rat subcutaneously or intranasally produced an antidiuretic response, whereas this dose orally and i ontophoretically did not induce the response (data not shown). The oral and i ontophoretic routes required a dose of 0.1 μg of DDAVP per rat to induce the antidiuretic response. The response to DDAVP following subcutaneous (0.02 μg/rat), intranasal (0.02 μg/rat), oral (0.1 μg/rat) and i ontophoretic administration (0.1 μg/rat) was prolonged for 5.8, 3.4, 1.3 and 3.5 h, respectively. These results indicate that the i ontophoretic delivery was 0.2 and 2.7

<table>
<thead>
<tr>
<th>Administration route</th>
<th>Dose</th>
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<tr>
<td>Subcutaneous</td>
<td>0.02 μg/rat (0.5 ml)</td>
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<tr>
<td>Intranasal</td>
<td>0.02 μg/rat (25 μl)</td>
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<tr>
<td>Oral</td>
<td>0.1 μg/rat (0.5 ml)</td>
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<td>10 μg/rat (0.5 ml)</td>
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<td>Iontophoresis (0.1 mA, 2000 Hz, duty 50%)</td>
<td>0.1 μg/rat (1.0 ml)</td>
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<td></td>
<td>1 μg/rat (1.0 ml)</td>
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<td></td>
<td>10 μg/rat (1.0 ml)</td>
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**Fig. 1. Antidiuretic Response to DDAVP Induced by Various Administration Routes**

A, subcutaneous administration (0.02 μg/rat); B, intranasal administration (0.02 μg/rat); C, oral administration (0.1 μg/rat); D, i ontophoresis for 12 h (0.1 μg/rat). Each point represents the mean ± S.D. of 3 rats.
times as effective as intranasal and oral administration, respectively. The rate of loss of response to DDAVP following iontophoretic delivery was similar to that following subcutaneous delivery. In contrast, the urine volume following intranasal delivery gradually and inconsistently returned to the critical level after decreasing to a minimal level (Fig. 1). Nishigaki et al. reported that plasma concentration of DDAVP showed twin peaks after nasal administration since DDAVP administered intranasally remained in nasal cavity and effused at gastrointestinal tract. The phenomenon suggests the varied rate of disappearance of plasma concentration and antidiuretic response because of inconsistent absorption. On the other hand, DDAVP after subcutaneous administration is absorbed more rapidly and consistent than that after intranasal administration. The pattern of the antidiuretic response by iontophoresis may be similar to that after subcutaneous administration since DDAVP is quantitatively delivered into a skin by electrical force.

DDAVP is usually administered intranasally, and physicians are aware of the difficulties of controlling polyuria when the nasal route is not available in patients with defective eyesight or frequent rhinitis, or in small children. DDAVP has biological activity when taken orally, and is clinically valuable in these patient groups. However, the availability of DDAVP delivered orally is very low, and the dose needed to treat diabetes insipidus patient by the oral route is approximately 20 times greater than by the nasal route. In the present study, pharmacological effect by iontophoresis was comparable to the nasal route in rats at a dose only five times higher than the nasal route dose, and was 2 to 3 times as effective as the oral route. This suggests that the iontophoresis could be valuable for DDAVP delivery in the treatment of neurogenic diabetes insipidus and nocturia patients.

**Relationship between the Dose of DDAVP and the Prolongation of Antidiuretic Response Delivered by Iontophoresis** The duration of the antidiuretic response to DDAVP delivered by iontophoresis increased with the increase of dose (Fig. 2). This result supports the suggestion by Saffran et al. that the dose–urine flow curve after a rectal administration of DDAVP was log-linear, and suggests that a definite relationship can be found between the dose of DDAVP and the prolongation of antidiuretic response following iontophoresis.

At a higher dose (10 µg of DDAVP per rat), the urine volume was lowered for about 14 h following iontophoretic delivery, whereas the urine flow was decreased for only 6 h following oral delivery at the same dose, and gradually returned to the critical level with large inter-subject variabilities (Fig. 3). The result supports that oral absorption of DDAVP is inconsistent because it is affected by enzymatic degradation in the gastrointestinal tract. Transdermal iontophoretic delivery was thus found to be superior to oral administration in the prolongation of the antidiuretic response to DDAVP at a higher dose.

**Effect of Duration of Current Application on Antidiuretic Response to DDAVP and % Remaining in the Donor** We examined the effect of the duration of current application on the prolongation of the antidiuretic response. The response to DDAVP was prolonged for about 9 h with the application of current for 1 h. The response was extended slightly with longer applications of iontophoresis (Fig. 4). We also examined the percentage of DDAVP remaining in the anodic donor. When pulsed direct current at 0.1 mA was passed for 1 h, DDAVP in the anodic
Fig. 4. Effect of Duration of Current Application on Antidiuretic Response to DDAVP at a Dose of 10 μg/rat
Each point represents the mean ± S.D. of 3–4 rats.

Fig. 5. Effect of Duration of Current Application on % of DDAVP Remaining in the Anodic Donor after Iontophoretic Delivery at a Dose of 10 μg/rat
Each point represents the mean ± S.D. of 3 rats.

The duration of current application was extended to 15 hours. The amount of DDAVP remaining in the donor decreased steeply to 48% of the initial dose, and then gradually decreased (Fig. 5). There was no detectable amount of DDAVP in the donor after 6 hours. These results suggest that the blood concentration rises higher level since DDAVP is steeply absorbed into the skin at 1 hour, and this results in a prolonged response to DDAVP. Despite absorption of most of DDAVP remaining in the donor in the subsequent iontophoretic treatment after 1 hour, the antidiuretic response to DDAVP was not extended as long as expected. This must be because low absorption rate does not contribute effectively to elevated blood concentration and prolonged response to DDAVP. We suggest that the antidiuretic response to DDAVP can be effectively controlled by regulating the absorption of DDAVP at the short-term iontophoresis rather than prolonged treatment.

In conclusion, the iontophoretic delivery of DDAVP was suggested to be an attractive alternative to oral and nasal routes from the standpoint of the prolongation of the antidiuretic response.

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REFERENCES