Safe and Efficient Transdermal Delivery of Desmopressin Acetate by Iontophoresis in Rats

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Iontophoretic delivery of desmopressin acetate (DDAVP) was assessed for delivery efficiency and drug stability, both in vitro and in vivo. The effect of current intensity and duration of current application on the decomposition of DDAVP was investigated in vitro. It was shown that when a current of 0.1 mA was applied for 5 min, the decomposition of DDAVP was negligible. In vivo experiments under the same conditions showed that the antidiuretic response to DDAVP persisted for about 6 h. Furthermore, when this iontophoresis was repeated 3 times at intervals of 4 h, the antidiuretic response persisted for about 11 h. These results suggest that repeated short-term iontophoresis is a safe and effective technique for transdermal delivery of DDAVP.

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

Iontophoresis has recently become a promising technique to enhance and control the delivery of molecules across the skin.1–6) This technique could be of particular benefit for the delivery of peptide or protein drugs, as oral delivery of these drugs can result in poor absorption or enzymatic degradation in the gastrointestinal tract.7,8) In a previous paper, we described transdermal delivery of desmopressin acetate (DDAVP), an antidiuretic nonapeptide, using iontophoresis.9) However, degradation of DDAVP was suspected when a longer current application was carried out. We then created an in vitro evaluation system, and confirmed that iontophoresis can cause DDAVP decomposition.9) Drug decomposition during iontophoresis has been reported by others,9,10) and is a major concern due to possible ill effects of decomposition products on a patient’s health. Nevertheless, few reports on in vivo iontophoresis have taken drug decomposition into consideration.

The purpose of this study was to find out the conditions of iontophoresis which enable a drug to be efficiently delivered transdermally without causing the drug to decompose. We used DDAVP as the model drug in this study. The electrochemical stability of DDAVP was evaluated in vitro without skin, to avoid the influence of the transdermal absorption. Furthermore, the pharmacological effects of DDAVP delivered by iontophoresis were monitored in vivo by measuring urine volumes in rats.

MATERIALS AND METHODS

Materials  DDAVP was supplied by Ferring AB (Malmo, Sweden). Isotonic sodium chloride solution and 5% aqueous glucose solutions 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, Japan), respectively. Sodium n-butanesulfonate was from Tokyo Kasei Kogyo Co. (Tokyo, Japan). All other reagents were obtained from Kanto Chemical Co. (Tokyo, Japan). Male Wistar rats, 8 to 10 weeks old, weighing 280 to 350 g, were from SLC (Shizuoka, Japan).

In Vivo Studies  Animal Preparation: The rats were anesthetized with 1 g/kg body weight of urethane by intraperitoneal injection. The anesthetized animals were fixed in the supine position on an operating table whose surface temperature was adjusted to 35–37 °C. A polyethylene tube (PE50, Clay Adams) was inserted and fixed in the femoral vein, and a DDAVP-free hypotonic solution (isotonic sodium chloride solution: 5% aqueous glucose: water = 27:36:37 by volume, for a total osmolality of 175±5 mOsm/kg) was continuously infused at a rate of 3.0 ml/h with a syringe pump (STC-523, 525, Terumo). After the ureter was bound up with a suture thread, a polyethylene tube (PE50, Clay Adams) was inserted into the urinary bladder and fixed with a suture thread. Urine was collected from the urinary bladder at intervals of 15 min to measure urine volume. After the stabilization of urine volume at about 1 ml every 15 min (generally after 16 to 24 h), the experiment was started.

DDAVP Solutions: DDAVP was dissolved in the hypotonic solution at a concentration of 20 ng/ml for i.v. bolus administration. For i.v. continuous infusion, DDAVP was dissolved in the hypotonic solution at concentrations of 0.005, 0.01, 0.02, 0.04 and 0.4 ng/ml. For iontophoresis, DDAVP was dissolved at a concentration of 10 μg/ml in a 3 mg/ml citric acid solution (adjusted to pH 6 with 0.1 N or 1 N sodium hydroxide).

DDAVP Administration: 1) i.v. bolus administration  After rats had equilibrated to the continuous DDAVP-free hypotonic solution infusion, 1 ml of DDAVP solution was injected into the infusion tube for a few seconds. Then, 1 ml of DDAVP-free hypotonic solution was injected in a few seconds in order to wash away the DDAVP remaining in the tube. The continuous infusion with DDAVP-free hypotonic solution was resumed.

2) i.v. continuous infusion administration  i.v. continuous infusion of DDAVP was carried out by switching DDAVP-free hypotonic solution over to DDAVP-containing hypotonic solution. The solution containing 0.4 ng DDAVP/ml was administered by continuous infusion at a rate of 3.0 ml/h for 16 h. The solutions containing 0.005, 0.01, 0.02 and 0.04 ng DDAVP/ml were infused at a rate of 3.0 ml/h for 6 h.

3) Iontophoretic administration  Before the start of the experiment, the abdominal region of the rat was carefully shaved with an electric clipper. Two silicone tubes (inner diameter: 10 mm, height: 30 mm) were placed perpendicular to the skin, 10 mm apart, on the shaven abdominal surface and fixed in place with an adhesive (Alon Alfa, Conishi Co.). A carbon electrode (diameter: ~8 mm, W-0901Q, Nilhon Koden)
was inserted into the tube to be used as a positive electrode, up to a height of about 5 mm from the skin surface. A platinum electrode (diameter: ~8 mm) was inserted into the tube to be used as a negative electrode, up to a height of about 5 mm from the skin surface. Each electrode was connected to an electrostimulator (SEM-3301, Nihon Koden) and an isolator (SS-302J, Nihon Koden). One ml of DDAVP solution (10 µg/ml) was placed into the positive electrode tube, and 1 mL of isotonic sodium chloride solution was placed into the negative electrode tube. A current application of 0.1 mA (pulsed current: 2000 Hz, duty 50%) was made with the electrostimulator.

Evaluation of Antidiuretic Response: The baseline urine volume was considered to be the mean of the four fractions immediately preceding DDAVP administration. The time during which urine volumes were lower than this baseline was considered to be the duration of antidiuretic response.

In Vitro Studies  Electrochemical in Vitro System: In the in vitro evaluation system, the effects of DDAVP concentration, current intensity and duration of current application on the electrochemical stability of DDAVP were investigated. A carbon electrode (as a positive electrode) and an AgCl electrode (as a negative electrode) were inserted into a chamber prepared with a silicone tube (i.d.: 10 mm, height: 30 mm). The AgCl electrode was prepared by coating Ag with a layer of AgCl electrolytically, in a solution of 4 N hydrochloride. One ml of DDAVP solution (3 mg/ml citric acid solution, adjusted to pH 6 with 1 N sodium hydroxide) with DDAVP at a concentration of 10, 50 and 100 µg/ml was placed into the chamber. An electrostimulator (SEN-7203, Nihon Koden) and an isolator (SS-403J, Nihon Koden) were used to apply a current of 0.1, 0.2 and 0.5 mA (2000 Hz, duty 50%) for 5, 15 and 30 min at a temperature of 23±1°C. Then the percentage of undegraded DDAVP remaining in the chamber was determined by HPLC analysis.

HPLC Analysis: After current application, 50 µl of the DDAVP solution in the chamber was analyzed by HPLC using a reverse phase chromatography column (Superspher 100 RP-125×4.0 mm, 4 µm, Merck). The mobile phase consisted of a mixture (75:13:12) of 0.07 M phosphate buffer (pH 5.2), acetonitrile, and methanol containing 0.8 mg/ml sodium n-butanesulfonate. The absorbance of the eluents at a wavelength of 220 nm was detected by a UV spectrophotometer. The percentage of undegraded DDAVP remaining in the chamber was calculated by comparing the DDAVP peak areas from the dosage solutions before and after current application.

RESULTS

Comparison between i.v. Bolus Administration and Continuous Infusion  The effect of the administration rate on the antidiuretic response to DDAVP was investigated in order to clarify the relationship between absorption rate and duration of pharmacological response. After DDAVP was administered by i.v. bolus injection at a dose of 20 ng/rat, urine volume was decreased for about 6 h (Fig. 1). However, following i.v. continuous infusion for 16 h (at a total dose of 20 ng/rat), a decrease in urine volume was observed for about 19 h (Fig. 2). Thus, because the duration of antidiuretic response was increased by administering the same dosage over a long period of time, a prolonged transdermal absorption of DDAVP would be desirable.

The Minimum Concentration of DDAVP Required to Induce Antidiuretic Response  The minimum concentration of DDAVP required to induce antidiuretic response by i.v. continuous infusion was investigated. At a DDAVP concentration of 0.005 ng/ml, there was little decrease in urine volume (Fig. 3). A concentration of 0.01 ng/ml produced a slight decrease in urine volume. At 0.02 ng/ml or higher concentrations, the urine volume was markedly decreased. These results suggest that even very low concentration of DDAVP (more than 0.01 ng/ml) absorbed into the skin may be sufficient to induce antidiuretic response.

Electrochemical Stability of DDAVP  The electrochemical stability of DDAVP at a concentration of 100 µg/ml was investigated with the in vitro system. Almost 100% of DDAVP was still present after at a current application of 0.1 mA for 30 min, whereas 3.8 and 5.0% of DDAVP was degraded after application of 0.2 and 0.5 mA for 30 min, respectively. Under the relatively stable conditions for DDAVP
(0.1 mA for 30 min), the effect of DDAVP concentration on the stability of DDAVP was investigated. The percentage of undegraded DDAVP remaining after current application decreased with decreasing initial concentrations of DDAVP (10 μg/ml: 92.4%, 50 μg/ml: 96.3%, 100 μg/ml: 99.1%). When the low concentration DDAVP solution (10 μg/ml) was treated by a current application of 0.1 mA for 5, 15 and 30 min, more DDAVP remained as the duration of current application was shortened (percentage of DDAVP remaining: 99.3, 94.9, and 92.4, respectively). This indicates that even if a low concentration of DDAVP is applied, the decomposition of DDAVP is negligible with a current of 0.1 mA for 5 min.

Iontophoresis Based on the results obtained above, in vivo iontophoresis using a solution containing 10 μg
DDAVP/mL and applying a current of 0.1 mA for 5 min was evaluated in the rat. This iontophoretic treatment induced a decrease of rat urine volume for about 6 h, as shown in Fig. 4. Since the percentage of undegraded DDAVP remaining in the donor solution was as high as 83.7%, the current application of 0.1 mA for 5 min was performed a total of 3 times at intervals of 4 h. A decrease in urine volume was extended to about 11 h by this repeated iontophoresis (Fig. 5).

DISCUSSION

In general, when a freeze-dried product is reconstituted before use or when medicines are combined in one container, stability and compatibility of these medicines before and during administration are established in order to avoid undesirable side effects. Stability of medicines during iontophoretic application should likewise be established.

We previously established an in vitro evaluation system to investigate the electrochemical stability of DDAVP. This system can avoid underestimation of degradation products caused by their absorption into skin, because both electrodes exist in the same chamber without skin. Furthermore, it can predict the decomposition of drug on the surface of positive electrode, because AgCl which is subject to electrolysis is used as a negative electrode. In the present study, we investigated the effect of current intensity, duration of current application and drug concentration on stability of DDAVP with this in vitro system. We found that the decomposition of DDAVP correlated linearly with duration of current application (r=0.977) in obedience to Faraday's law of electrolysis. The result suggests that a mechanism of DDAVP decomposition is caused by removal of electrons by an anode, and thus the duration of current application affects the amount of DDAVP decomposition. Consequently, a short current duration is desirable in order to minimize DDAVP decomposition during in vivo iontophoresis. The present study has revealed that DDAVP can be safely administered by iontophoresis because a sufficient amount of DDAVP was absorbed across the rat skin in vivo, under conditions that cause very little degradation (0.7%) of DDAVP (0.1 mA for 5 min).

We previously reported that when a 10 μg dose of DDAVP was iontophotically delivered to a rat by application of a 0.1 mA current for 60 min, a decrease in urine volume was observed for about 9 h. In the present experiment, when the same current intensity and DDAVP dose was delivered by applying a current for 5 min every 4 h a total of 3 times, the decrease in urine volume persisted for about 11 h, despite a net current duration of only 15 min (Fig. 5). These differences must be due to the various absorption profiles of DDAVP following these iontophoretic treatments. Wu et al. presented the following three distinct patterns of drug sensitivity: (a) dose- and time-dependent, (b) time-dependent and (c) time-independent. DDAVP is classified as type (b). In the present study, the antidiuretic response to DDAVP lasted longer by i.v. continuous infusion than by i.v. bolus administration at an equivalent dose. Moreover, the antidiuretic response was induced by continuous infusion at a very low concentration (0.01 ng/mL). Therefore, a slower, more prolonged application of DDAVP, even at low concentrations, would be expected to induce a longer pharmacological response. Thus, the repeated application of current spread out over hours must more closely approximate a prolonged application of DDAVP than a continuous current application, for the same total time of current application.

In conclusion, repeated short (5 min) 0.1 mA iontophoretic applications of DDAVP induces a long-lasting antidiuretic response with minimal electrolytically-induced DDAVP decomposition.

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REFERENCES