Effect of Pulse on Iontophoretic Delivery of Desmopressin Acetate in Rats

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The effect of pulse parameters (duty and frequency) in a constant direct current iontophoresis on the antidiuretic response (elevation in rat urinary osmotic pressure) of desmopressin acetate (DDAVP) was examined in diabetes insipidus rats. Although antidiuretic response was not affected by frequency, it was induced by a duty of more than 26% and prolonged with increasing duty. A positively relationship between dose and AUC; the area under the osmotic pressure–time curve, was confirmed by intravenous administration of DDAVP, and the AUC induced by the iontophoretic delivery increased with increasing duty. The voltage across rat skin required to maintain a constant current density was investigated. A higher voltage was initially applied rat skin in a higher duty. This was related the prolonged pharmacological response induced by iontophoresis.

Key words iontophoresis; desmopressin acetate; pulse current; skin permeability

The skin, particularly the stratum corneum, is a very efficient diffusion barrier,1 and thus drugs which are appropriate candidates for a passive transdermal delivery system are restricted.2,3 For other drugs the use of chemical enhancers, which act by altering in skin properties, can lead to undesirable side effects.4,5 In recent years, systemic drug delivery by iontophoresis has gained much attention, as it may be able to avoid the limitations of passive transdermal delivery.6–8 The use of iontophoresis obviates the need for chemical enhancers and eliminates the problems associated with their presence. Iontophoresis enhances transdermal drug delivery by the flow of an electric current, producing electroosmosis within a solvent stream.9–11 Although iontophoretic delivery is normally carried out with a simple direct current (DC), the use of pulsed DC has been suggested as a method to obtain higher drug flux and lower skin damage since the impedance of skin decreases with the increasing frequency of the applied current or voltage.12,13 Based on this theory, several studies have reported the efficiency of a pulse waveform in constant-current or constant-voltage iontophoresis.14–16 However, few investigators have mentioned the amount of drug absorption and a prolonged effect.

In a previous paper17 we found that variations in amperage and in the duration of short-term iontophoresis under a constant current can manipulate percutaneous absorption of desmopressin acetate (DDAVP). The purpose of the present study was to investigate (1) the efficiency of a pulse on the iontophoretic delivery of DDAVP and (2) the relationship between the pulse and the pharmacological response profile. Studies were carried out on rats with diabetes insipidus, and the pharmacological effect was monitored by measuring urinary osmotic pressure.

MATERIALS AND METHODS

Materials DDAVP was supplied by Ferring AB, Malmö, Sweden. All other reagents were obtained from Kanto Chemical Co. Inc., Tokyo, Japan.

In Vivo Study In vivo percutaneous absorption studies were performed using a diabetes insipidus model. Male Wistar rats (360–400 g) were injected with a hypotonic solution. Changes in urinary osmotic pressure were monitored as an indicator of percutaneous absorption of DDAVP.18,19 Each experiment was carried out with three rats.

Urethane was intraperitoneally administered at 1 g/kg for anesthesia. The abdominal surface was then shaved using an electric clipper and the animals were fixed to a thermostatically regulated plate at 35 to 37 °C. The urinary tract (penis) was closed with a string and the urinary bladder was exposed with a 1 cm incision through the abdomen. The bladder was cannulated with a polyethylene tube (PE50, Clay Adams) to collect urine specimens. Before each iontophoresis experiment, a hypotonic solution consisting of 1.18 g NaCl and 9.0 g glucose in 500 mL 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 urinary osmotic pressure 80 to 120 mOsm/kg. The solution was maintained at 37 °C and injected at a constant rate of 0.2 ml/min in all experiments. About 0.1 to 2.0 ml of urine was collected every 10 min in order to check the osmotic pressure by an osmometer (OSM-1, Shimadzu Co.). Baseline was established by pre-injecting 500 pg/kg DDAVP at a concentration of 200 pg/ml into the femoral vein. The antidiuretic effect in each animal was evaluated to confirm sensitivity to DDAVP. When no response was shown, the experiment was terminated. After recovery of urinary osmotic pressure to the control level, iontophoretic delivery of DDAVP was evaluated by fixing with adhesive (Aron Alpha, Conishi Co.) two cylindrical-type iontophoretic applicators (made of silicone, 10 mm i.d., 30 mm height) 10 mm apart on the cleanly shaven abdomen.

DDAVP solution (10 μg DDAVP in 1 ml of pH 6.0 citric acid–disodium hydrogen phosphate buffer solution, ionic strength 0.1) and 1 ml of 0.9% NaCl 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). A current density of 0.2 mA/cm² (quantity of electricity 0.12 coulomb/cm²)
Table 1. Summary of Iontophoresis Conditions

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Duty $^a$ (%)</th>
<th>Duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DC $^b$</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>2000</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>2000</td>
<td>50</td>
</tr>
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<td>4</td>
<td>2000</td>
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<tr>
<td>5</td>
<td>500</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>10000</td>
<td>50</td>
</tr>
</tbody>
</table>

$a$) Duty shows on/on+off ratio. $b$) DC shows simple direct current.

was delivered to the platinum electrodes. The area surrounded by time and urinary osmotic pressure, which was corrected by subtracting a base line, (AUC$_{\text{i.e.}}$) was calculated. The other conditions for this study are summarized in Table 1. Voltage across the rat skin was periodically monitored throughout the experiment by oscilloscope (VC-11, Nihon Koden Co.) and thermal array recorder (RTA1200, Nihon Koden Co.).

**Relationship between Dose and Antidiuretic Response**

500, 1000 and 1500 pg/kg DDAVP were injected into the femoral vein using the same diabetes insipidus rat. Urine was collected every 10 min in order to check the osmotic pressure. AUC$_{\text{i.e.}}$, the area under the osmotic pressure-time curve, was calculated.

**RESULTS**

**Relationship between Dose and Antidiuretic Response**

Relationship between dose and AUC$_{\text{i.e.}}$, was estimated by intravenous administration (i.v.) of DDAVP. When DDAVP was injected to the same rat at dosage of 500, 1000 and 1500 pg/kg, the AUC$_{\text{i.e.}}$ increased with increasing dose and was closely related to the dose (Fig. 1: correlation coefficient $r = 0.998$).

**Effect of Pulse on Antidiuretic Response**

The effectiveness of current duty (on/on+off ratio) and frequency in delivering DDAVP was studied with a duty range of 26—100% and a frequency range of 500—10000 Hz, respectively. The antidiuretic response was induced by a duty of more than 26% (Fig. 2). The same maximum osmotic pressure was obtained by a duty more than 50%. The response was transient at a duty 25 and 50% and prolonged at a duty of 74 and 100%. AUC$_{\text{i.e.}}$, induced by iontophoresis was calculated. The AUC$_{\text{i.e.}}$, increased with increasing the duty (AUC$_{\text{i.e.}}$, at the duty of 25, 50, 75 and 100% were 6920, 59887, 97351 and 109442 min·Osm/kg, respectively). Frequency did not affect the transdermal delivery of DDAVP in maximum response and AUC$_{\text{i.e.}}$ (Fig. 3).

**Voltage Drop Across the Rat Skin**

The voltage drop required to maintain a current density of 0.2 mA/cm² iontophoresis was measured. The initial voltage ($V_0$) increased with increasing duty ($V_0$ at the duty of 25, 50, 75 and 100% was 5.5, 7.1, 8.9 and 9.1 V, respectively). On the other hand, each voltage profile induced by iontophoresis with various duties decreased as a function of time during the experiment (Fig. 4). In the case of simple DC (a duty of 100%), the voltage was initially high voltage though extremely decreasing with the passage of time. Although the decrease in voltage at a duty of 50 and 74% was similar in slope of the curve, voltage at a duty of 74% was higher than that at a duty of 50%. Voltage at a duty of 26% was approximately constant and low.
DISCUSSION

A therapeutic response vs. time profile may be used directly for drug absorption analysis.20,21 DDAVP has a potent antidiuretic response, and the antidiuretic activity is measured by urine osmolality.18 A positive relationship has been reported between DDAVP intranasal or oral doses and biological response.22,23 This is reflected by a clear relationship between dosage and plasma DDAVP levels. We also found in our study that AUC was closely related to the dose. On the basis of the result, we monitored changes in urinary osmotic pressure as an indicator of percutaneous absorption of DDAVP and estimated the effect of pulse on the iontophoretic delivery of DDAVP using the AUC.

Other investigators have reported the relationship between drug absorption and pulse in iontophoretic delivery.14,15 But it is not clear which the effect of pulse would be investigated on total drug absorption and prolonged response because the drug delivery was estimated by a maximum response and plasma concentration. In the present study, we found that a higher duty yielded the better absorption (AUC) in the iontophoretic delivery of DDAVP, and a skin was initially subject to a higher voltage in duties of 74 and 100%, at which the antidiuretic response was prolonged, despite difference in the change of voltage between both duties. On the other hand, the voltage was relatively low in a lower duty (26 and 50%) in which a prolonged response was not observed (Figs. 3, 4).

Hinsberg et al.24 have reported that variations of frequency or duty cycle do not result in significantly different peptide transport rates, and the flux of peptides appears to be controlled by the applied voltage. On the other hand, it has been reported that the cutaneous depot is formed during iontophoresis and modulates entry of drug into the systemic circulation.25 It is speculated in those reports that a higher voltage increases the flux of drug and forms a large depot, which controls a venous efflux. In the present study, the depot may be generated by an initially high voltage at a higher duty.

In conclusion, a higher duty is available for obtaining a prolonged pharmacological effect in a short time application of iontophoresis, and a lower duty is preferred for the control of the drug absorption with iontophoresis duration.

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REFERENCES