Electrochemical Stability of Desmopressin Acetate during Iontophoresis

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The electrochemical stability of desmopressin acetate (DDAVP) was investigated. After a direct current of 1 mA was passed through two electrodes chosen from platinum, silver and silver chloride, the decomposition of DDAVP was observed. The degradation products of DDAVP yielded with a Pt(anode) and AgCl(cathode) pair were similar to those observed in an anodic donor after *in vivo* iontophoresis. Although the Ag(anode)/Pt(cathode) degradation pattern of DDAVP was similar to that of an *in vivo* cathodic donor HPLC chromatogram, it was different from that of the anodic donor. In the anodic system, the stability of DDAVP was independent of the pH, and decreased with increasing concentration of NaCl.

Key words iontophoresis; desmopressin acetate; stability

Desmopressin acetate (DDAVP) is a derivative of vasopressin, and has an antidiuretic action. Intranasal administration of DDAVP has been studied as a convenient and efficient method of drug delivery.[1-3] However, conventional transmembrane delivery depends on passive diffusion and produces large inter-subject variabilities. Iontophoresis has recently become a promising technique to enhance and control the delivery of drug molecules across the skin.[4-11] We have studied iontophoretic transdermal delivery of DDAVP in a previous paper,[12] and we reported that variations in amperage and in the duration of short-term iontophoresis under a constant direct-current can control the duration of pharmacological response and reduce variability of absorption. However, the drug electrochemically decomposed during iontophoresis.[13-15]

The efficiency of drug delivery can be affected by the electrode materials and drug counterions employed in an iontophoretic system.[14] It is preferable to use a silver (Ag) electrode with chloric ion (Cl^-) in the donor because this combination results in little or no change in the donor pH. However, with an Ag electrode, it is difficult to control drug delivery in a prolonged iontophoresis, because Ag is easily oxygenated, and Ag^+ released from the electrode precipitates on the anode surface as silver chloride (AgCl) during iontophoresis. The iontophoretic drug delivery can be also carried out with a stable electrode (Pt),[11,16] which needs to be investigated on the electrochemical stability of drug.[15] But the quantitative estimation of a drug's decomposition across a synthetic membrane and skin is difficult, because the drug and its degradation products migrate from the donor to membrane, skin and receptor.

The objective of the present study was to investigate the stability of DDAVP using a system which can predict a drug's decomposition during iontophoresis, without skin or membrane. Studies were carried out by *in vitro* system with two electrodes chosen from Pt, Ag and AgCl, and the stability of DDAVP was evaluated by HPLC.

Materials and Methods

Materials Desmopressin acetate (DDAVP) was supplied by Ferring AB (Malmo, Sweden). All other reagents were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan).

***In Vitro* System for Studies of Electrochemical Stability** Two electrodes of Pt, Ag or AgCl wire (1 mm dia.) were assembled by coiling the first 30 mm, and immersing in a cylindrical-type device (silicone, 10 mm i.d.). The AgCl electrode was prepared by coating Ag electrolytically in a solution of 4 N hydrochloride (HCl), with a layer of AgCl.[17] The electrodes were connected to the electrostimulator (Fig. 1). The combinations of various electrodes are summarized in Table 1.

Electrolysis in the *in Vitro* System One milliliter of 154 mM sodium chloride (NaCl) was placed in the above system and a constant current of 1 mA was passed through the system for 5 min at 25 °C. The pH of the NaCl solution was measured before and after each experiment.

Study on Electrochemical Stability of DDAVP *In Vitro* System: DDAVP solutions (100 μg DDAVP in each 1 ml of 20 mM citric acid solution with and without 154 mM NaCl) were placed in the *in vitro* system. The solutions were adjusted to pH 6 with 0.1 N sodium hydroxide (NaOH). The combination of Pt(anode)/AgCl(cathode) and Ag(anode)/Pt(cathode) as the electrodes were used. Decomposition of DDAVP was evaluated at 1 mA, 25 °C for 30 min. DDAVP and its degradation products were analyzed by HPLC using Superspher 100 RP-18, 125 × 4.0 mm, 4 μm. The mobile phase consisted of a mixture (75 : 13 : 12)

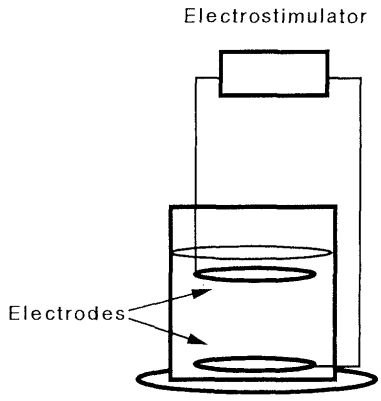

Fig. 1. *In Vitro* System for Determination of Electrochemical Stability of Desmopressin Acetate

Table 1. pH Changes of Sodium Chloride Solution to Current Passage with Various Electrodes

		Combination of electrode		
		Pattern 1	Pattern 2	Pattern 3
Anode		Pt	Ag	Ag
Cathode		AgCl	Pt	AgCl
pH	1 mA, 0 min	6.0	6.0	6.0
	1 mA, 5 min	3.3	11.3	5.9

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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 using an UV-detection at 220 nm wavelength. The samples were analyzed in triplicate.

In Vivo Study: The *in vivo* study was performed using male Wistar rats (300—400 g). Urethane was intraperitoneally administered at 1 g/kg for anesthesia. The abdominal surface was then shaved using an electric clipper. The stability of DDAVP was evaluated by fixing with two cylindrical type iontophoretic applicators (made of silicone, 10 mm i.d., 30 mm height) adhesive (Aron Alpha, Conishi Co.) 10 mm apart on the cleanly shaven abdomen. DDAVP solutions were placed in the anode applicator and the cathode applicator. Platinum electrodes, (8 mm dia.) were immersed in each applicator at about 5 mm from the skin surface. After iontophoresis was conducted at 3 mA for 60 min using an electric stimulator, DDAVP and its degradation products were then analyzed by HPLC.

Effect of NaCl: DDAVP solutions (100 μg DDAVP in each 1 ml of 20 mM citric acid containing 0, 20, 77 and 154 mM NaCl adjusted to pH 6 with 0.1 N NaOH) were placed in the *in vitro* system with Pt(anode)/AgCl(cathode) as the electrodes. Decomposition of DDAVP was evaluated at 1 mA, 25 °C for 30 min. DDAVP and its degradation products were then analyzed by HPLC.

Effect of pH: DDAVP solution (100 μg DDAVP in each 1 ml of 20 mM citric acid) was placed in the *in vitro* system with Pt(anode)/AgCl(cathode). The solution was adjusted to pH 4, 6 and 8 with 0.1 N NaOH, compared with no adjustment (pH 2.5). A direct current of 1 mA was passed at 25 °C for 30 min. The pH was measured after experiments.

Results

Electrolysis in the *in Vitro* system The pH of the NaCl solution to current passage with two electrodes chosen from Pt, Ag and AgCl was measured (Table 1). After a constant direct-current of 1 mA was passed through the *in vitro* system with the combination of Pt(+)/AgCl(−), the pH of the NaCl solution decreased remarkably. In the case of Ag(+)/Pt(−), the pH increased remarkably. In the case of Ag(+)/AgCl(−), the pH of the solution did not change.

Study on the Electrochemical Stability of DDAVP DDAVP solutions were placed in the *in vitro* system with the electrodes in combinations of Pt(+)/AgCl(−) and Ag(+)/Pt(−). A constant direct-current of 1 mA was passed through these electrodes and DDAVP and its degradation products were then analyzed by HPLC. HPLC chromatograms are shown in Fig. 2 (A, B) and Fig. 3 (A, B). The chromatograms in Fig. 2-A and Fig. 2-B were obtained using Pt(+)/AgCl(−) in the citric acid solutions without and with NaCl, respectively. DDAVP (main peak) and the characteristic four unknown degradation products (D1, D2, D3 and D4) were detected in the solution without NaCl (Fig. 2-A). In contrast, the degradation product (D4) and many products in the front area of the chromatogram (except the main peak) were detected in the solution with NaCl (Fig. 2-B). The chromatograms in Fig. 3-A and Fig. 3-B were obtained by Ag(+)/Pt(−) in the solutions without and with NaCl, respectively. The main peak and another four unknown degradation products (D5, D6, D7 and D8) were detected in the solution with NaCl (Fig. 3-B). No degradation products were detected in the solution without NaCl (Fig. 3-A). Aggregation was observed in the solution at the same time.

After *in vivo* iontophoresis at 3 mA, DDAVP and its degradation products in the anodic donor and cathodic donor were analyzed by HPLC. HPLC chromatograms of these donor solutions are shown in Fig. 2-C and Fig. 3-C. In the citric acid solution, the independent four-degradation products observed in Fig. 2-A and Fig. 3-B were detected in the anodic donor and cathodic donor, respectively. The *in vitro* system with Pt(+)/AgCl(−) was remarkably similar to the *in vivo* anodic iontophoresis in the HPLC pattern. Conversely, the HPLC pattern in the case of Ag(+)/Pt(−) was similar to the *in vivo* cathodic iontophoresis rather than the *in vivo* anodic iontophoresis.

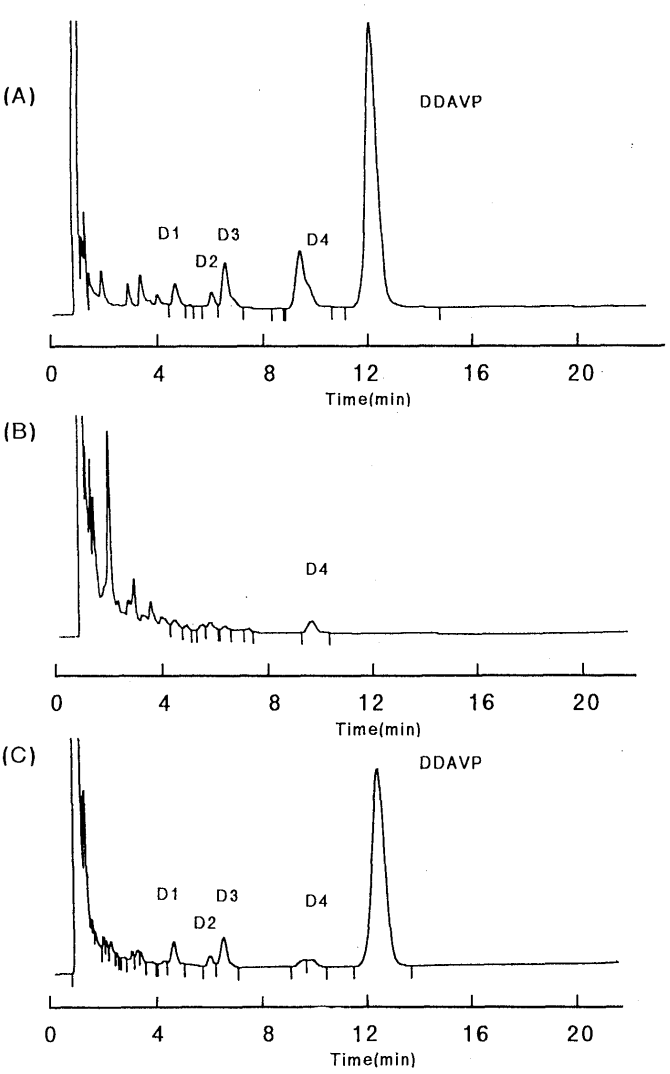

Fig. 2. HPLC Chromatograms of the *in Vitro* System with Pt(+)/AgCl(−) and the *in Vivo* Anodic Iontophoresis

(A) *in vitro* system without NaCl, (B) *in vitro* system with NaCl, (C) *in vivo* anodic iontophoresis.

Effect of NaCl The effect of NaCl on the electrochemical stability of DDAVP in citric acid solution was investigated with Pt(+)/AgCl(−) (Fig. 4). The stability of DDAVP was decreased with increasing concentrations of NaCl. When 154 mM NaCl was added, DDAVP was completely decomposed.

Effect of pH The effect of pH on the electrochemical stability of DDAVP in citric acid solution was estimated with Pt(+)/AgCl(−). The pH slightly decreased after these experiments. However, no difference in the stability of DDAVP was observed in the pH range of 2.5—8.0 (1.8—6.8 after each experiment) (Fig. 5).

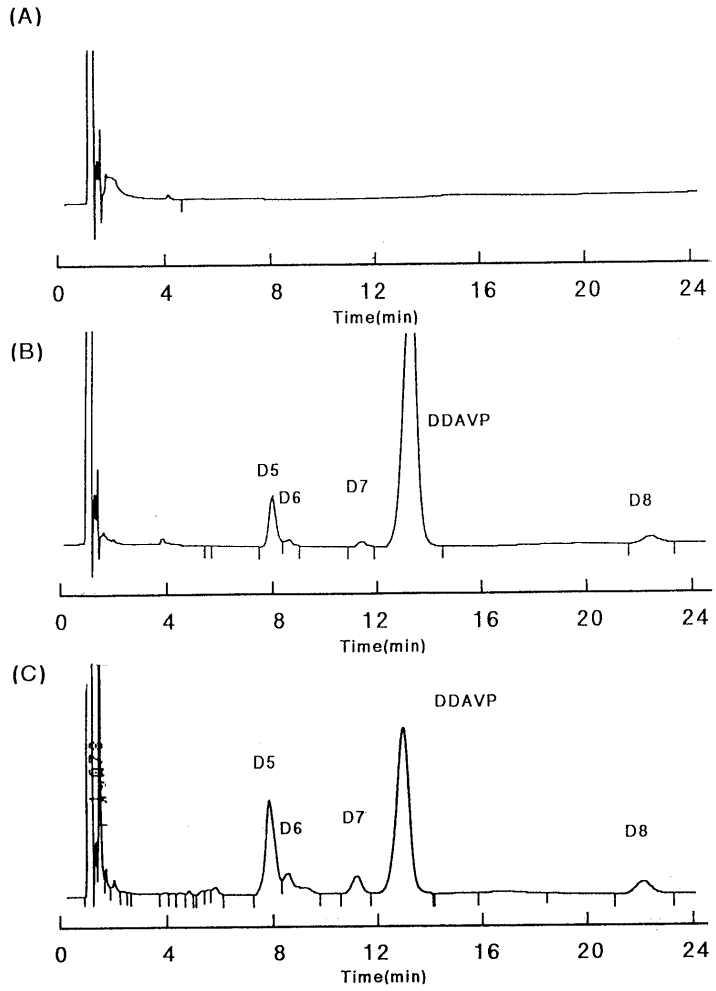

Fig. 3. HPLC Chromatograms of the *in Vitro* System with Ag(+)/Pt(−) and the *in Vivo* Cathodic Iontophoresis

(A) *in vitro* system without NaCl, (B) *in vitro* system with NaCl, (C) *in vivo* cathodic iontophoresis.

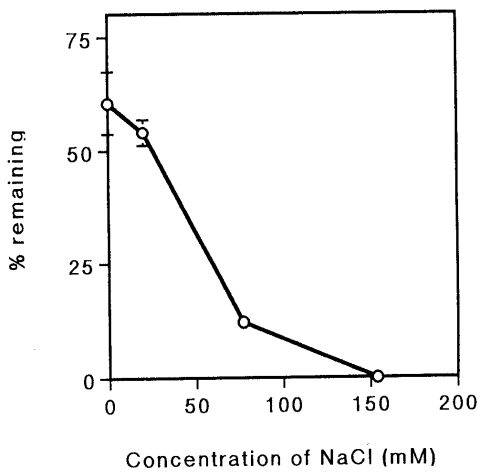

Fig. 4. Effect of NaCl on Electrochemistry of DDAVP in the *in Vitro* System with Pt(+)/AgCl(−)

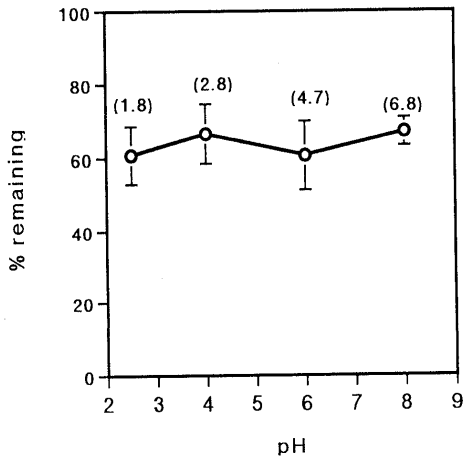

Fig. 5. Effect of pH on the Electrochemical Stability of DDAVP in the *in Vitro* System with Pt(+)/AgCl(−)

Each value in parentheses represents pH after experiments.

Discussion

Phipps *et al.* have reported that during iontophoresis, electrochemical oxidation and reduction occur at the anode and cathode, respectively, and selection of the anode or cathode material, in combination with selection of an appropriate drug counterion, can significantly reduce the electrochemical alteration in the donor compartment.[14] In their report, when a LiCl solution and a Pt anode were placed in the donor compartment, water was oxidized to form oxygen gas and hydronium ions during the iontophoresis. This reaction caused the pH decrease in the donor solution. In the combination of a $LiNO_3$ solution and a Ag anode, the primary oxidation reaction which occurred during iontophoresis is $Ag \rightarrow Ag^+ + e^-$, and silver ions are released into the donor solution, since silver

oxidizes at a lower anodic potential than water. In contrast to these reactions, the donor compartment containing LiCl solution and a silver anode does not generate pH change, because a released silver ion precipitates on the anode surface as AgCl ($Ag + Cl^- \rightarrow AgCl + e^-$).

In our present *in vitro* study, the pH in the 154 mM NaCl solution decreased in the combination of Pt anode and AgCl cathode (Table 1-pattern 1) and increased in the combination of Ag anode and Pt cathode (Table 1-pattern 2). The results must be caused by electrochemical oxidation and reduction of water at the Pt anode and the Pt cathode, respectively. If a drug has a lower redox potential than water, the drug will be subject to a redox reaction at a Pt electrode.

We investigated the electrochemical stability of DDAVP using an *in vitro* system and compared the system with the stability of DDAVP after *in vivo* iontophoresis. After *in vivo* iontophoresis at 3 mA, the HPLC pattern observed in the *in vitro* system with electrodes of Pt/AgCl and Ag/Pt was remarkably similar to that observed in the anodic donor and the cathodic donor, respectively. This suggests that the *in vitro* system can reproduce the redox reaction generated by anode and cathode in the *in vivo* iontophoresis. In general, electrolysis is affected by a potential difference. In the present study, the potential difference applied in the *in vitro* system did not necessarily agree with that in the donor of the *in vivo* iontophoresis (data not shown). An adjustment of potential differences in the *in vitro* system may enable us to quantitatively analyze the stability of a drug during iontophoresis.

In the *in vitro* system with Pt(+)/AgCl(−), DDAVP was not detected in the HPLC chromatogram by adding NaCl (Fig. 2-B). On the other hand, we found that DDAVP content decreased with increasing concentrations of NaCl, as shown in Fig. 4. It has been reported that tissue injury occurred as the result of chlorine liberation at the anode, the basis for which is chloride ion oxidation.[18] Chlorine, into which chloride ions are electrochemically transformed, may drastically decompose the peptide (DDAVP). This suggests that if NaCl is added in the anodic donor during iontophoresis (for example, in order to modulate ionic strength,[19,20] a drug will be destabilized.

However, in the case of Ag(+)/Pt(−), no peaks were detected in the solution without NaCl (Fig. 3-A) and the aggregation was noted in the solution. When Ag is used as the anode without NaCl, silver ions are released into the solution.[14] It is known, by the silver-staining method for detecting proteins and peptides in polyacrylamide gels, that the silver ion has a strong affinity for many proteins and peptides.[21,22] We suggest that the aggregation observed in the present solution may be produced by the denaturation of DDAVP bonded to silver ions. Our result suggests that the cathodic system with Ag(+)/Pt(−) is applicable when excess NaCl is permitted into the system.

Seth *et al.* have reported that the magnitude of change in pH is dependent on the stability of hydrocortisone during iontophoresis,[15] but we found in the present system that pH was independent of the stability of DDAVP.

In conclusion, we observed that our new system, which need not use skin, can investigate the stability of a drug during *in vivo* iontophoresis. Our results suggest (1) the possibility of destabilizing a drug by adding NaCl, and (2) the pH independency on stability of the drug.

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