IONTOPHORETIC TRANSDERMAL DELIVERY OF ANGIOTENSIN II AND ITS ANTAGONISTS

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ABSTRACT

Angiotensin II and its antagonists were used as permeant probes to investigate the mechanism of transdermal iontophoretic delivery of peptide/protein drugs and compare the effect of hydrophobic difference between the peptides drugs on the skin permeability. Transport of angiotensin II and its antagonists through excised rabbit pinna skin were enhanced with iontophoresis by monophasic periodically pulsed current, amplitude=1mA, frequency=1000Hz and on/off ratio equals 50%. The delivery rate was proportional both to the current intensity and pulse duration of the stimulation. Results showed that the permeation rate of [Sar, Ile] Angiotensin II > [Sar, Ala] Angiotensin II > Angiotensin II. Iontophoretic mobility and hydrophobicity were the key factors dominating the transdermal iontophoretic delivery rate of angiotensin II and its antagonists.

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1. INTRODUCTION

Iontophoresis is a method of drug delivery that uses an electric current to drive charged molecules, even uncharged molecules, across biologic membranes. Iontophoretic transdermal delivery is a noninvasive method which minimizes trauma, risk of infection, and damage to the skin. When a drug is administered by topical iontophoresis, the drug bypasses the digestive tract and escapes first-pass metabolism in the liver [1,2]. Effective use of iontophoresis depends upon the chemical properties of the drug. The drug must be electrically charged or in a solution sufficiently ionized so as to carry a measurable current in excess of 10 (-10) A at physiologic pH. Utilization of drugs with molecular weights exceeding 800 to 12,000 results in an uncertain rate of delivery [3]. However, research is underway investigating the possibility of transporting

peptides and proteins (such as insulin) by iontophoresis.

Charged molecules and ionic drugs, even neutral molecules, can be driven increasingly through skin into the body under an electrical potential, either in continuous mode or pulsed mode. Iontophoretic transdermal drug delivery is a noninvasive method. It has potential advantage for long-term medication whereas needle insertion may cause anxiety, pain and may increase the risk of infection. For peptide/protein drug delivery, this technique is a promising method to provide a better way of administration because injection is always employed nowadays.

Besides, peptide/protein drugs become increasingly important in medication, the alternative administrations other than injection are gaining more attention. Iontophoretic transdermal delivery provides an attractive method for peptide delivery because it is noninvasive, avoids chemically hostile gastrointestinal environment, avoids hepatic elimination at first-pass. It also provides a controlled and/or sustained delivery, even in a pulsatile manner, which is critical for the desired physiological response of some hormonal drugs.

Green et al.[4] showed that normalized flux following cathodal iontophoresis of anionic amino acids were independent of lipophilicity, but might be in-

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versely related to molecular weight. For zwitterionic amino acids and neutral tripeptides, despite the large variation in lipophilicity, normalized flux is relatively constant. But we don't think the relationship is so simple. Turner et al., [5] anodal iontophoresis of poly-L-lysines (polycations) demonstrated inverse dependence of iontophoretic delivery upon the MW of penetrant and might have a phenomenon of cut-off MW. They also showed that the electroosmotic flow (anode to cathode) would be inhibited even shut down by polycations. Angiotensin 2 and its antagonists were chosen to illustrate the feasibility of transdermal iontophoretic delivery because they may be regarded as stable model molecules for peptide delivery and their molecular weight were about 1000, near the upper threshold of detectable passive permeation.

Clemessy et al., [6] found that under the current density of 0.28 mA/cm^2 , the angiotensin II transport was enhanced 20-fold in comparison to passive permeation. The enhancement of permeation of peptide drugs by iontophoresis was proportional to the current density [2,6,7]. But the real mechanism of transport is still not clear even ambiguous [5,8].

In our early study, we had investigated the possibility of transdermal iontophoretic delivery of the smaller peptide, TRH (M.W.=362) [2]. Though there wasn't any detectable amounts of drug being permeated through excised skin during passive diffusion, the permeation amount of TRH increased with respect to time when an electrical field was applied. The permeation flux was proportional to the applied current density during iontophoresis. However, the factors affecting the iontophoretic permeation rate and the real mechanism of transport is still not clear. To investigate the feasibility and the mechanism of transdermal iontophoretic delivery of larger peptides, the transport pathways of peptide/protein drugs during iontophoresis and the effects of hydrophobicity/hydrophilicity of drugs, angiotensin II and its antagonists were used as a permeant probe. The molecular weight of angiotensin II and its antagonists were about 1000 dalton, almost near the critical molecular weight of passive/active permeation margin. Moreover, angiotensin II was regarded as a stable model molecule for peptide delivery [6].

2. MATERIALS AND METHODS

Angiotensin II [Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, Mw.=1046.2] and its antagonist [Sar, Ala] angiotensin II [Sar-Arg-Val-Tyr-Ile-His-Pro-Ala, Mw. 926.1] and [Sar, Ile] angiotensin II [Sar-Arg-Val-Tyr-Ile-His-Pro-Ile, Mw. 968.2] (Sigma, USA) were used as model drugs for comparison. Other chemicals (reagent grade, Sigma, USA or Wako Chemicals, Japan) were used as received unless otherwise stated. Water was obtained

from a purification system. Rabbit inner pinna skin were used in all experiments as its transdermal permeation characteristics are similar to human cadaverous skin (8). Skin was freshly excised from New Zealand white rabbits, about 3-5 kg, obtained from our animal center in the College of Medicine. The outermost layers of skin (epidermis skin) were taken from the animals immediately after they were sacrificed and were used as soon as possible.

2.1 Determination of iontophoretic flux in vitro

The basic experimental arrangement is depicted in Figure 1. Iontophoretic diffusion experiments in vitro were made in the Valia-Chien side-by-side diffusion cell (model VSC-1, Crown Glass, Somerville, NJ), with a controlled rate of stirring (600 rpm) in both half cells. Fresh skin was placed between the cells. An isothermal condition at $37 \pm 0.2^\circ \text{C}$ was maintained in both half-cells by circulating water of controlled temperature through a water jacket. An orifice (0.69 cm^2) connected the two diffusion cells. The volume of each cell was 3.5 mL. Angiotensin II and its antagonists with the same concentration, 10mg/ml respectively, were poured into the donor compartment. Platinum wires (99.99 % purity; length 2 cm $\times$ diameter 0.5 mm) served as electrodes. The electrodes were connected to a universal power source, (HP 3245A, Hewlett-Packard, Palo Alto, CA), that output a periodically pulsed or constant current.

2.2 Determination of Angiotensin II and Its antagonists

Angiotensin II and its antagonists were assayed by capillary zone electrophoresis (P/ACE System 2100, Beckman). UV detector at 214 nm was used and the temperature of the capillary was maintained at $30 \pm 0.1^\circ \text{C}$. The separation buffer contained phosphate (pH 7.4) at an ionic strength 0.02M. The sample was injected into the column under pressure for 5 s, corresponding to approximately 30 nL. All separations were performed

Fig. 1 Experimental setup.

for 10 min at a constant voltage 15kV. The retention time of Angiotensin II and its antagonists was about 9.90 and 8.84 min respectively. The sample signals were analyzed with software (Beckman System Gold, version 8.1).

2.3 Thermal analysis of skin

The thermal behavior of skin before and after iontophoresis was recorded using a differential scanning calorimeter (DSC2910, TA, New Castle, DE). The heating rate was 10 °C/min over a temperature range of 20-200°.

3. RESULTS AND DISCUSSION

Different mode of current could be used to enhance the permeation rate of drugs through the excised skin, either with a continuous mode of current or with a monophasic periodically pulsed current. A continuous mode of current may cause electrochemical polarization in the skin that impedes the drug to penetrate. While monophasic periodically pulsed current will depolarize the reverse electric field established between the skin. Depolarization of the skin during iontophoresis with pulsed current resulted in a significantly increased amount of transdermal delivery. In this study, monophasic periodically pulsed current was used.

In a degradation test, the change of angiotensin 2 was monitored when angiotensin 2 was contacted with skin and deteriorated under the experimental condition: periodically monophasic-pulse current, amplitude=1mA, frequency=1000Hz and on/off ratio equals 50%. During the experimental time interval, no significant change of angiotensin 2 was found. In some cases silver/silver chloride electrode would cause serious precipitation of peptides/proteins in iontophoresis [9,10]. While the electrode made of brass or steel (medical grade) would reveal small amounts of copper, nickel and chromium [11] which are well known dermal allergies. Platinum electrodes were used in this study. This inert electrode resulted in a shift of pH and gas bubbling from electric hydrolysis of water and concomitant production of H⁺ and OH⁻ ions.

Figure 2 shows that the cumulative permeation amount of angiotensin II and its antagonists with and without facilitated by an external electrical field. For angiotensin II and its antagonists, undetectable amounts of drug are transported across excised skin during passive diffusion (without facilitated by an external electrical field). In our early study, the smaller peptide, TRH (MW.=362), also didn't show any detectable amounts of drug permeated through excised skin during passive diffusion [2]. While during iontophoresis, under the monophasic periodically pulsed current with the frequency of current: 1000Hz, duty cycle 50%, offset 1mA, and the mean current 1 mA, the permeation

amount of angiotensin II and its antagonists increased with respect to the applied current. The cumulative amount of [Sar, Ala] angiotensin II was linearly increased as duration of iontophoresis increased. According to linear regression, the correlation coefficient is R²=0.968.

The flux of angiotensin II and its antagonists, J_i , is attributed to three mechanism of transport--passive diffusion, electrically induced motion of ions, and electroosmotic convection, represented in Equation 1.

$$J_i = J_{ip} + J_{ie} + J_{iv}$$

$$J_i = -D\nabla C_i - \frac{Dz_i F C_i}{RT} \nabla \phi + J_{iv} \quad (1)$$

As experimental results show that J_{ip} approaches zero and according to Pikal's model, $J_{iv} = L_{ve} \nabla \phi$, the flux is proportional to only the applied electrical field, $\nabla \phi$.

$$J_i = -\frac{Dz_i F C_i}{RT} \nabla \phi - L_{ve} \nabla \phi \quad (2)$$

Experimental results showed that the resistance of the skin barrier soon approaches a steady value. As a result, the flux of angiotensin II and its antagonists is directly proportional to the total applied current density. The permeation flux of angiotensin II and [Sar,Ala] angiotensin II were 3 and 12 $\mu\text{M}/\text{cm}^2\text{hr}$ respectively.

From Figure 2, we also found that after the current shut down, the passive permeation of angiotensin II and [Sar,Ala] angiotensin II were slightly increased compared to that of before iontophoresis, the flux from almost zero (undetectable) to 0.3 $\mu\text{M}/\text{cm}^2\text{hr}$. That meant that the skin structure was slightly changed possibly

Fig. 2 Cumulative permeation amount of angiotensin II and its antagonists during monophasic periodically pulsed iontophoresis. 1 mA, for 3 hours.

Fig. 3 Skin DSC.

due to the effect of iontophoresis or overhydration. From the DSC diagram of skin before and after iontophoresis (Figure 3), it confirmed that skin structure was slightly changed because the endothermal peak flattened. The structure of skin was loosely organized after iontophoresis as shown by low heat flow compared to the original one before iontophoresis. Overhydration of the skin during iontophoresis may be the major reasons.

Clemessy et al., [6] found that electroosmotic flow was more important in contribution to the iontophoretic transdermal delivery of peptides. Most of the current during iontophoresis was carried by buffer ions or electroosmotic flow, not due to the drug charge itself. Green [4] also considered the electroosmotic flow was more important. But if the convection term were the only factor dominating the transdermal permeation flux during iontophoresis, the permeation flux of angiotensin and its antagonists would be much the same in our experiments due to the same permeation condition. But actually, there have significant difference in the iontophoretic flux at the same permeation condition. At the current density of 0.28 mA/cm^2 , the H_2O flux gains 4-fold increase during iontophoresis, while for angiotensin II, 20-fold increase. From the correlation between angiotensin II flux versus H_2O flux, Clemessy et al., [6] thought that iontohydrokinesis could be responsible for a significant part of the transport of angiotensin.

From the experimental results, Hatanaka et al., [12] suggested that the convective flow markedly contributed to the total skin permeability of a hydrophilic drug. In the visualization of iontophoretic pathways, Turner and Guy [5] found the larger molecules would accumulate within the pore section of hairfollicles.

Therefore, for iontophoretic transdermal delivery of hydrophilic drug, such as peptides, the major path-

Fig. 4 CE resisting time of angiotensin II and its antagonists in phosphate buffer under 15 kV electrophoresis.

ways would be the pore channels within the skin. Considering these pore channels being composed of many of capillaries, the skin permeability can be related to the electrophoretic mobility of each capillary channel.

$$\mu_{ep} = \frac{v_{ep}}{E} = \frac{L_d / t_m}{V / L_t}$$

If the electroosmotic flow is the most important factor governing the iontophoretic delivery, the electroosmotic flow capability can be expressed by the following equation

$$v_{eo} = \frac{\Xi \zeta}{4\pi\eta} E$$

The electrophoretic mobility of angiotensin II and its derivatives can be simulated by the capillary electrophoresis of these compounds. Figure 4 show the retention time of different derivatives of angiotensin 2 and itself. Under the condition pH 2, 25mM phosphate buffer and 200 mM Tween 20. The electrophoretic mobility sequence of angiotensin II and its derivatives are

[Sar, Ala] angiotensin II > [Sar, Ile] angiotensin II > angiotensin II.

The retention time of [Sar, Ala] angiotensin II, [Sar, Ile] angiotensin II, and angiotensin II are 20.3, 22.9 and 29.6 min respectively [13]. Because in the capillary electrophoresis, the major factors affecting the iontophoretic mobility are the molecular size and charges and in the phosphate buffer under 15 kV electric field, the mobility of angiotensin II was slower than its antagonists, [Sar, Ala] angiotensin II and [Sar,Ile] angiotensin II (Figure4). The main reason may due to the different degree of dissociation of angiotensin II and its antagonists. From Table 1, we can see the charge of [Sar, Ala] angiotensin II and [Sar,Ile] angiotensin II are great than angiotensin II. At pH 7.4,

Table 1. Physical properties of angiotensin II and its antagonists.

Drug	Structure	M.W.*	P _I value	Charge		
				+2	+1	0
Angiotensin II		1046.2	7.7	1.8-4.5	4.5-6.7	6.7-10
[Sar, Ala]AT2*		926.1	9.9	2.3-6.7	6.7-9.8	9.8-10
[Sar,Ile]AT2*		968.2	9.9	2.4-6.7	6.7-9.8	9.8-10

*AT2: Angiotensin II ; M.W.:Molecular weight.

Table 2 Partition coefficients of angiotensin II and its antagonists.

Drug	Structure	Partition coeff. Log P _{octanol/water}
Angiotensin II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe	-1.86
[Sar, Ala]AT2*	Sar-Arg-Val-Tyr-Ile-His-Pro-Ala	-2.82
[Sar,Ile]AT2*	Sar-Arg-Val-Tyr-Ile-His-Pro-Ile	-1.03

the dissociation of angiotensin II was quite low. Most of the angiotensin II were in the neutral state. While the [Sar, Ala] angiotensin II and [Sar,Ile] angiotensin II are almost with same degree of dissociation. But the molecular size of [Sar, Ala] angiotensin II was a slight smaller than that of [Sar,Ile] angiotensin II, the iontophoretic mobility of [Sar, Ala] angiotensin II is slightly higher than that of [Sar,Ile] angiotensin II.

Except the charge and size effects of peptide molecules, the hydrophobicity of peptide molecules also play a key factor affecting the iontophoretic transdermal permeation rate of peptides. Green et al., (1992, J. Control. Rel. 21:187-190, 1992) showed that the permeability of anionic Asp of the model peptide $\text{CH}_3\text{-CO-Ala-X-Ala-NH-C(CH}_3\text{)}_3$ was highly lower than that of neutral amino acid residual. So the the iontophoretic permeation of angiotensin II ([Asp,...,Phe]) was lower than that of [Sar, Ala] angiotensin II and [Sar,Ile] angiotensin II. The hydrophobicity of angiotensin II and its antagonists can be represented by the partition coefficient of peptides in octanol/water. The partition coefficient of [Sar, Ala] angiotensin II, [Sar, Ile] angiotensin II and angiotensin II were calculated from Abraham and Leo [14] and tabled in Table 2. The hydrophobicity of angiotensin II and its antagonists are as follow.

[Sar, Ala] angiotensin II > [Sar, Ile] angiotensin II > angiotensin II.

Amino acid of sarcosine [Sar] in the angiotensin II antagonists is more hydrophobic than the amino acid of aspartic acid [Asp] in the angiotensin II. Including the charge and size effects, the angiotensin II moved

most slowly, i.e. lowest electrophoretic mobility. So the lowest skin permeability.

4. CONCLUSIONS

Iontophoresis is a promising method to enhance permeation of peptides through skin. Transport of a model peptide drugs (angiotensin II and its antagonists) through excised rabbit pinna skin was enhanced with iontophoresis. The resulting flux enhanced by iontophoresis in the steady state was proportional to the applied current density and pulse duration. The delivery rate was proportional both to the current intensity and pulse duration of the stimulation. For the same current density, results showed that the permeation rate of [Sar, Ile] Angiotensin II > [Sar, Ala] Angiotensin II > Angiotensin II. Iontophoretic mobility and hydrophobicity were the key factors dominating the transdermal iontophoretic delivery rate of angiotensin II and its antagonists. Iontophoretic mobility can be simulated by the species mobility in capillary electrophoresis. And hydrophobic peptides permeated more quickly as compared with the hydrophilic peptides with similar primary structure and charge.

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