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Iontophoretic Transport of Donepezil Hydrochloride through Skin: Flux Enhancement by Chemical Enhancer and Iontophoresis

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ABSTRACT − The objective of this work is to investigate the effect of chemical enhancer and current on the flux of donepezil hydrochloride (DH) through skin. Ethanol and N-methyl pyrrolidone (NMP) were used as chemical enhancers in combination with iontophoresis. We also have studied the effect of pH on flux and evaluated the role of electroosmosis. In vitro flux study was performed at 33°C, using side-by-side diffusion cell and full thickness hairless mouse skin. Passive flux of DH without enhancer was very small. As the concentration of enhancer increased, passive flux increased. After current application, flux increased markedly and the time to reach maximum decreased. Without enhancer, maximum flux was about 50 fold larger than that obtained without current. These results indicate that electromigration is playing a major role for the transport. As the enhancer concentration increased, flux also increased. NMP and ethanol increased not only the passive delivery, but also the iontophoretic delivery. Flux results indicate that ethanol has better ability than NMP in enhancing the transport of DH. The magnitudes of increase in flux by these enhancers indicate that there is a large synergistic effect in flux enhancement. Flux results from pH study showed that electroosmotic flow is reversed at low pH and the flux is hindered. These results provided some information on the flux enhancing ability of ethanol and NMP in combination with iontophoresis. The data also provided some mechanistic insights into the role of electromigration and electroosmosis on flux through skin.

Key words − Iontophoresis, Donepezil hydrochloride, N-methyl pyrrolidone, Ethanol, pH

According to the recent demographic study in South Korea, more than 10% of the total population is elderly people, and expected an Aged Society with elderly people more than 14% in the near future(Jhoo et al., 2008). Actually, this trend with aged people occupying higher % of the population is prevailing throughout the world and the number of patients with degenerative neurologic disease such as Parkinson's or Alzheimer's disease is increasing. Alzheimer's disease is especially drawing focus around the world due to its rapid increase in number of patients (~ 5 million/year), and it has been predicted that the number of patients with Alzheimer's will reach 100 million by 2050 (Brookmeyer et al., 2007; England, 2006).

Alzheimer's disease is the most common type of dementia occupying more than 50% of different types of dementia such as vascular dementia, dementia with Lewy bodies, and frontotemporal demential (Querfurth et al., 2010). A deficit in central cholinergic transmission caused by degeneration of the basal forebrain nuclei is an important pathological and neurochemical feature of Alzheimer's disease (Scarpini et al., 2003). Accumulation of beta-amyloid proteins creates tangle within the neuron, causes abnormal protein accumulation, and eventually leads to brain cell death. In this process, the amount of neurotransmitter called acetylcholine decreases (Francis et al., 1999; Lesne et al., 2006; Zhao et al., 2008). A cure for this disease is not yet found and, currently, the treatment for Alzheimer's disease is simply delaying the progress of memory loss, cognitive disorder, and behavioral disturbances (Jann et al., 2002). Acetylcholine esterase (AchE) inhibitors such as Aricept® (DH), Reminyl (Galantamine hydrobromide), Cognex® (Tacrine) and Exelon® (Rivastigmine tartarate) are used for the drug treatment (Lleo et al., 2006). Rivastigmine (Exelon) is widely used after receiving FDA's approval in 2007 (Cummings et al., 2007). N-methyl d-aspartate (NMDA) receptor antagonist such as Namenda® (Memantine) is also used as an effective drug treatment (Olivares et al., 2011). The mode of action of Memantine is to block a messenger chemical known as glutamate, which is released in excess amount when brain cells are damaged by Alzheimer's disease (Tariot et al., 2004).

DH has been used for patients with mid and moderate Alzheimer's disease(Nakano et al., 2001). It is a centrally acting piperidine derivative (Figure 1), which non-competitively and reversibly inhibits acetylcholinesterase, highly selective for acetylcholinesterase, and binds to plasma proteins (96%) (Tiseo et al., 1998). Donepezil is primarily metabolized by

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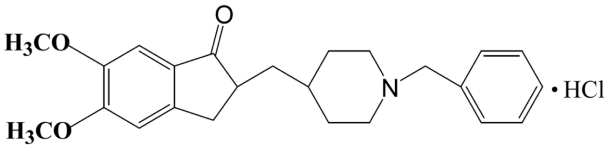

Figure 1. Chemical structure of donepezil hydrochloride (M.W. 415.96).

CYP2D6 and 3A4 and undergoes extensive first-pass metabolism (Tiseo et al., 1998). It prevents reduction of acetylcholine (cerebrum neurotransmitter) by selectively suppressing AchE which degrades acetylcholine (Jann et al., 2002). It is absorbed in the gastrointestinal tube when orally administered, reached the maximum blood concentration after 3 to 4 hours (Physician's, 1999). Approximately 72% of the dosage is excreted through the kidney, half-life is ~70 hours, and therapeutically effective concentration is known to be 30 to 75 ng/mL(Crismon, 1998; Kishnani et al., 1999). It is usually administered orally, but various side effects such as nausea, emesis, memory loss, and dyslogia are reported (Rogers et al., 1996).

Among different administration methods of drug for Alzheimer's patient, transdermal patch may provide various advantages such as reduced gastrointestinal disturbances, bypassing hepatic first pass effect and higher patient compliance (Subedi et al., 2010). Because one patch can deliver drug molecules for several days, it can reduce the burden of family members who is administering the medicine to patient everyday. It may also remove the difficulties for patients with swallowing problems of the tablet dosage form. Because the patch is applied externally on skin, it can help to tell whether the medication has been done or not. Rivastigmine patch (Exelon) has been developed and the efficacy, safety and tolerability were compared with conventional rivastigmine capsules and placebo in patients with Alzheimer's disease (Winblad et al., 2007). Once a week transdermal patch for donepezil has also been developed, though it is not yet approved by FDA for market (FDA, 2011).

In this study, we have investigated the flux enhancement of DH, using iontophoresis and chemical enhancers. Iontophoresis is a physical technology that enhances drug transport, using an electrical current across the skin (Yan et al., 2005). The two main mechanisms of transdermal iontophoresis include electromigration and electroosmosis (electric field induced solvent flow), and manipulation of the magnitude of current delivered and/or formulation parameters can allow the control of transdermal delivery (Leboulanger et al., 2004). In this work, we used ethanol and N-methyl pyrrolidone (NMP) as chemical enhancers in combination with iontophoresis to test whether there is a synergistic effect on flux. We also have studied the effect of pH on flux. Using these results, we have evaluated the role of electromigration and electroosmosis on the flux under different enhancer concentration and pH.

Materials and Methods

Materials and instruments

All reagents and chemicals were of the highest commercially obtainable purity. DH was obtained from Korea United Pharm. Inc. (Seoul, Korea). NMP, sodium phosphate monobasic and sodium phosphate dibasic were purchased from Sigma-Aldrich Co. (Seoul, Korea). Polyethylene Oxide (PEO, NF grade, M.W. 200,000) which was used to prepare the hydrogel was purchased from Union Carbide Corporation (Danbury, CT, USA). Buffer solutions were prepared using distilled water from Nanopure ultrapure water system D11921 (Barnstead, Iowa, USA). HPLC grade acetonitrile, ethanol and sodium chloride were obtained from Duksan Chem. (Seoul, Korea). The instruments used were as follow : power supply (Model PT 70-10 MDC, Power Tech (Ansan, Korea), Incubator (SI-900, Jeio Tech, Ansan, Korea), pH meter (model 320, Corning, New York, USA), HPLC (Futecs NS-4000 Daejeon, Korea), Deep freezer(Model 925, Forma Scientific Inc., Ohio, USA), Ultrasonicator (Model 5210, Branson, Danbury, CT, USA), Laser (CICU-F, Ilooda Co., Suwon, Korea).

Preparation of electrodes

Rod-shaped Ag/AgCl electrode and plate-shaped $Sn/SnCl_2$ electrode were used in this work. Ag/AgCl electrodes were used for their stability and reversibility. The rod-shaped Ag/AgCl electrodes were prepared by dipping the tip of an Ag wire (99.9%, Aldrich, Milwaukee, WI) (1 mm diameter) into the molten AgCl. The plate-shaped $Sn/SnCl_2$ electrode was prepared by covering the Sn particle (Duk-San Scientific Corp, Seoul, Korea) at 30 μm thickness using Knife doctor (Mitutoyo, Kawasaki, Japan) on polyester film, and by oxidizing in acidic condition.

Preparation of hydrogel

PEO hydrogel was prepared by adding PEO to aqueous solutions of different enhancer (NMP, ethanol) concentrations (enhancer : buffer solution = 0:10, 1:9, 2:8, 3:7, 4:6, v/v) until the concentration of PEO was 8% (w/w). As the aqueous solution, 128 mM phosphate buffer solution containing 4.8 mM NaCl (pH 6.0 or 2.5) and DH was used. Hydrogel were stored in refrigerator for 24 hour before use.

Drug assay

DH concentration was analyzed using high-performance liquid chromatography (HPLC).[25] The column was a Atlantis 5 μm ODS2 (4.6×150 mm) of Waters (Milford, MA, USA). Oven temperature was 33°C. The mobile phase was mixture of 20 mM phosphate buffer and acetonitrile (60:40) with flow rate of 1.0 mL/min. Detection wavelength was set at 315 nm, and injection volume was 50 μL.

***In vitro* permeation**

In vitro permeation study was performed at 33 incubator (Jeio Tech, SI-900, Ansan, Korea), using full-thickness hairless mouse skin. The skin was excised and frozen immediately after sacrifice of 8 week old mouse (Orient bio Inc., Seongnam, Korea). It was stored at -70°C and then was thawed just before use. A side-by-side diffusion cell (Yuil Science, Pusan, Korea) was used. The chamber held a volume of 1 mL and was magnetically stirred. For the study of the effect of enhancer, hairless mouse skin was mounted on side-by-side diffusion cell, and as the donor phase, PEO hydrogel (100 μL) prepared using pH 6.0 buffer solution and the enhancer was applied on skin. This amount of hydrogel contained 1 mg of DH. The area of skin exposed to permeation was 0.5 cm^2. Plate-shaped electrode ($Sn/SnCl_2$) was placed to cover the hydrogel. The diffusion cell (receptor phase) was filled with 128 mM phosphate buffer (pH 7.4) containing 4.8 mM NaCl, and rod-shaped Ag/AgCl electrode and magnetic bar were inserted into the cell (Figure 2). For the study of the effect of pH, PEO hydrogel (100 μL) prepared using different buffer solution (pH 6.0 or 2.5) was applied on skin. The diffusion cell (receptor phase) was filled with 128 mM phosphate buffer (pH 7.4) containing 4.8 mM NaCl. At a predetermined time interval, sampling was made and an equal volume of 1 mL buffer solution was added to the receptor chamber. Anodal current was applied and the current density was 0.4 mA/cm^2.

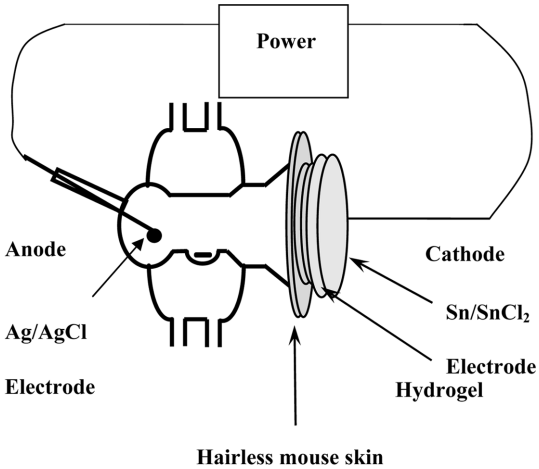

Figure 2. Schematic diagram showing iontophoretic delivery of DH from PEO hydrogel.

Results and Discussion

In our previous work, we have evaluated various factors which affect the iontophoretic transdermal transport, such as electrode polarity, current density, drug concentration and current profile (Choi and Oh, 2010). Hydrogel containing 8% poly(ethylene oxide) (PEO) showed the highest flux and was chosen as the hydrogel for further studies. Under experimental condition, DH was stable. Anodal delivery showed much larger flux than cathodal and passive delivery, possibly due to the positive charge of DH at pH 7.4. The results indicated that it was possible to deliver clinically effective amount of DH using iontophoresis. The data also provided some insight into the role of electromigration and electroosmosis during the transport through skin. In this work, we further investigated the effect of pH and chemical enhancers on iontophoretic permeation through skin. The effect of pH was studied in order to investigate the role of electroosmosis on flux. As chemical enhancers, we used NMP and ethanol in combination with iontophoresis to test whether there is any synergistic effect on flux. Because donepezil (pKa 8.90) is positively charged in neutral and acidic conditions, and based on the results from our previous study) (Choi et al., 2010), only anodal delivery is carried out. Passive delivery was also conducted to compare the results.

NMP is one of the main pharmaceutical cosolvent, and it acts as a very strong solubilizing agent (Avdeef, 2007). NMP has been used as a permeation enhancer for a various hydrophilic and hydrophobic drug molecules (e.g. insulin and estradiol) (Ahad et al., 2009; Koizumi et al., 2004). Using differential scanning calorimetry analysis, the mode of action for NMP was suggested as the increased partition of NMP into both keratin region and intercellular lipid domain, and thus alter the structure of keratin and lipid fluidity (Barry, 1987). Increased partition of NMP may increase the partition and diffusivity of drug molecules. The solvation around the polar head groups of the lipid and thus loosen lipid packing was also suggested as a possible action (Barry, 1987; Williams et al., 2004).

Ethanol is also used in many transdermal patches to enhance the flux of a various hydrophilic and hydrophobic drug molecules (e.g. 5-flurouracil and estradiol) (Pershing et al., 1990; Yum, 1994). Permeation enhancing activity of ethanol is probably achieved through various mechanisms, such as increased solubility of the drug in the vehicle, the alteration of the sol-

ubility properties of the tissue by the permeation of ethanol into the stratum corneum and a modification of thermodynamic activity of the drug in the formulation by the rapid evaporative loss of this volatile solvent (Barry, 1987; Megrab et al., 1995).

Effect of NMP

Flux results by passive diffusion from hydrogel containing different ratio of NMP to buffer (1:9, 2:8, 3:7 and 4:6) are shown in Figure 3(a). Control study using hydrogel without NMP was also carried out for comparison. Without NMP, flux was about 1.2 μg/cm^{2}hr in average during the whole duration of study. As the concentration of NMP increased, flux increased and reached maximum value after 5-7 hours later.

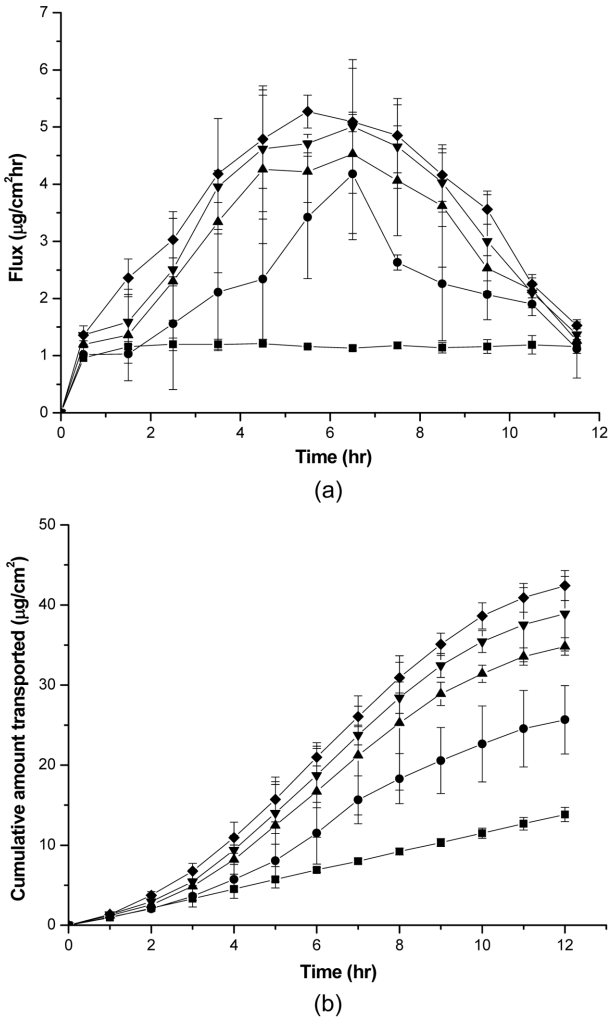

Figure 3. (a) Flux and (b) cumulative amount transported by passive delivery across hairless mouse skin *in-vitro*. PEO hydrogel containing NMP of different ratios (NMP : buffer = 1:9, 2:8, 3:7 or 4:6) was used. The amount of DH in the hydrogel was 1.0 mg. Each data point represents the mean (±S.D.) of 3 experiments. ■: 0:10 (control), ●: 1:9, ▲: 2:8, ▼: 3:7, ◆: 4:6.

When the ratio of NMP to buffer was 4:6, maximum flux was 5.3 μg/cm^{2}hr, which is about 5 fold larger than the value obtained without NMP. After the maximum value, flux decreased to 1.5 μg/cm^{2}hr at 11.5 hour, which is close to the value obtained without NMP. This bell shaped figure was also observed in other NMP to buffer ratios. Figure 3(b) shows the cumulative amount delivered for 12 hours. Control study without NMP showed 13.8 μg/cm^2 of cumulative transport after 12 hours. When the ratio of NMP to buffer was increased gradually to 1:9, 2:8, 3:7 and 4:6, cumulative amount transported increased from 26, 35, 39 and 42 μg/cm^2, respectively. These results indicate that NMP can act as a penetration enhancer for DH. As already discussed above, NMP acts as a very strong solubilizing agent and increased partition of NMP into both keratin region and intercellular lipid domain was suggested as the possible mode of action as a penetration enhancer (Barry, 1987). Increased partition of NMP may increase the diffusivity of donepezil molecules through the stratum corneum. It can also increase the partition of donepezil molecules into the stratum corneum, thus increase the flux. It is not clear why the flux profile is showing a bell-shaped figure. Considering the fact that the amount of DH transported is only a fraction of the amount in donor phase, depletion of DH in donor phase is not the reason. One possible explanation is the depletion of NMP content in the hydrogel with time, which may decrease the content of NMP in the stratum corneum. The extraction of NMP from stratum corneum into the receptor phase with time may also contribute to the depletion of NMP in the stratum corneum. Further study on the transport of NMP seems necessary for a better understanding on this point.

Flux with anodal current is shown in Figure 4(a). Control study without NMP was also carried out for comparison. After current application, flux increased markedly and the time to reach maximum decreased, when compared to those results from passive experiment. This result indicates that electromigration is playing the major role for the transport. Without NMP, maximum flux was achieved in 4 to 5 hours, and the magnitude was 53 μg/cm^{2}hr. This is about 50 fold larger than that obtained without current. This result indicates that donepezil is positively charged in donor solution, and anodal current is effectively enhanced the flux of DH. As the ratios of NMP to buffer are increased to 1:9, 2:8, 3:7 and 4:6, maximum flux increased gradually to 52, 70, 76 and 84 μg/cm^{2}hr, respectively. When the ratio was 4:6, maximum flux was 84 μg/cm^{2}hr. This is about 16 fold increase when compared with the maximum flux obtained without current (5.3 μg/cm^{2}hr). Flux maximum reached at about 5 hours after the current application, and after that, flux decreased with time. Figure 4(b)

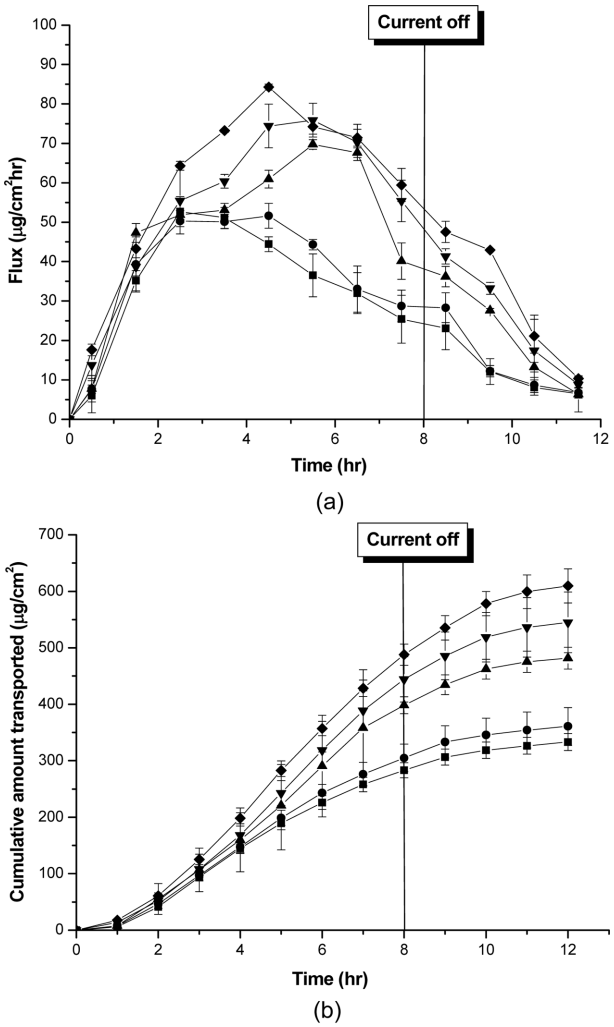

Figure 4. (a) Flux and (b) cumulative amount transported by anodal delivery across hairless mouse skin *in-vitro*. PEO hydrogel containing NMP of different ratios (NMP : buffer = 1:9, 2:8, 3:7 or 4:6) was used. The amount of DH in the hydrogel was 1.0 mg. Anodal current with current density of 0.4 mA/cm^2 was applied for 8 hours, and passive delivery was studied for 4 more hours. Each data point represents the mean (±S.D.) of 3 experiments. ■: 0:10 (control), ● : 1:9, ▲: 2:8, ▼: 3:7, ◆: 4:6.

shows the cumulative amount delivered for 12 hours. Control study without NMP showed 333 μg/cm^2 of cumulative transport after 12 hours. When the ratio of NMP to buffer was increased to 1:9, 2:8, 3:7 and 4:6, cumulative amount transported increased further to 361, 482, 545 and 610 μg/cm^2, respectively.

Flux by iontophoresis can be described by the following equation (Heit et al., 1997).

$$J_t = -D_i\left(\frac{dC_i}{dX} + \frac{C_i Z_i F}{RT}\frac{dV}{dX}\right) + VC_i$$

$$J_t = J_{diffusion} + J_{electromigration} + J_{electroosmosis}$$

where J is flux, F is Faraday constant, D is diffusion coefficient, C is concentration of ioninc drug molecule, Z is the charge of the ion, T is absolute temperature, V is the potential applied to the skin and R is the Boltzmann constant. This equation indicates that total flux by iontophoresis is the sum of passive diffusional flux by concentration gradient, electromigrative flux by electrical potential gradient and the electroosmotic flux due to the permselectivity of skin. Because donepezil (pKa 8.90) is positively charged in donor phase where pH is 6.0, electro-repulsive force between DH and anode results in this marked increase in flux. Electroosmotic volume flow also contributes to the flux, because the direction of electroosmotic volume flow is from anode to cathode.

After 8 hours of current application, current was off and the flux through skin was investigated for 4 more hours (Figure 4(a)). Without current, flux decreased rapidly and reached 10.3 μg/cm^{2}hr at 11.5 hour, when the ratio between NMP and buffer was 4:6. For NMP to buffer ratio of 1:9, 2:8 and 3:7, flux at 11.5 hour were 6.7, 6.3 and 8.7 μg/cm^{2}hr. These passive flux values are much larger than that observed in Figure 3(a) (1.2 μg/cm^{2}hr) at 11.5 hour. These results indicate that current application can affect the skin, and thus increase the passive flux. The bell shaped figure in flux was also observed in this transport experiment with current. As already suggested for the passive flux data, depletion of NMP in the stratum corneum could be a possible explanation for this. The decrease in concentration of DH in the donor hydrogel with time could also contribute to the decrease in flux. One more possible explanation is the decrease in electroosmotic flow, due to the decreased level of water in the donor hydrogel. These data, together with the passive flux data, clearly show that NMP increases not only the passive delivery, but also the anodal iontophoretic delivery. The magnitude of increase in iontophoretic flux by NMP also indicates that there is a synergistic effect in flux enhancement.

Effect of ethanol

Flux results by passive diffusion from hydrogel containing different ratio of ethanol to buffer (1:9, 2:8, 3:7, and 4:6) are shown in Figure 5(a). Without ethanol, flux was about 1.2 μg/cm^{2}hr in average during the whole duration of study. As the concentration of ethanol increased, flux increased and reached maximum value after 5-7 hours later. When the ratio of ethanol to buffer was 4:6, maximum flux was 5.6 μg/cm^{2}hr, which is about five times larger than the value obtained without ethanol. After the maximum value, flux decreased to 1.5 μg/cm^{2}hr at 11.5 hour, which is very close to the value obtained without ethanol (1. 2 μg/cm^{2}hr). This bell shaped figure was also

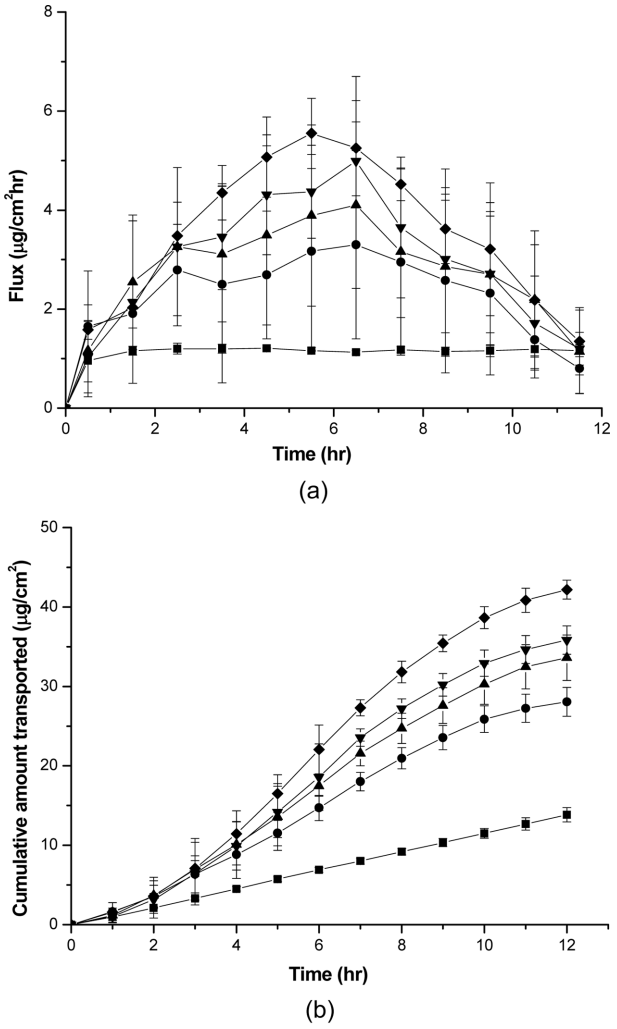

Figure 5. (a) Flux and (b) cumulative amount transported by passive delivery across hairless mouse skin *in-vitro*. PEO hydrogel containing ethanol of different ratios (ethanol : buffer = 1:9, 2:8, 3:7 or 4:6) was used. The amount of DH in the hydrogel was 1.0 mg. Each data point represents the mean (±S.D.) of 3 experiments. ■: 0:10 (control), ●: 1:9, ▲: 2:8, ▼: 3:7, ◆: 4:6.

observed in other ethanol to buffer ratios. These flux results are very similar to those results obtained for NMP in their magnitude and shape. Figure 5(b) shows the cumulative amount delivered for 12 hours. Control study without ethanol showed 13.8 μg/cm^2 of cumulative transport after 12 hours. When the ratio of ethanol to buffer was increased gradually to 1:9, 2:8. 3:7 and 4:6, cumulative amount transported increased to 28, 34, 36 and 42 μg/cm^2, respectively. These results indicate that ethanol can act as a penetration enhancer for DH. As already discussed above, permeation enhancing activity is probably due to the increase in solubility of drug in the stratum corneum (Megrab et al.,1995). Partition of ethanol may also increase the diffusivity of donepezil molecules through the stratum corneum. Lipid extraction from stratum corneum could be playing some role in enhancing activity, but the concentration and amount of ethanol used in this work were too low for lipid extraction (Bommannan et al., 1991). As already mentioned for the results of NMP, it is not clear why the flux profile is showing a bell-shaped figure. Because the amount of DH transported is only a fraction of the amount in donor phase, depletion of DH in donor phase is not the reason. One possible explanation is the depletion of ethanol content from the hydrogel with time, which may decrease the transport of ethanol into the stratum corneum. The extraction of ethanol from stratum corneum into the receptor phase with time may also contribute to the depletion of ethanol in the stratum corneum. Further study on the transport of ethanol seems necessary for a better explanation.

Flux with anodal current is shown in Figure 6(a). Control study without ethanol showed marked increase in flux. This result indicates that electromigration is playing the major role for the transport. As the ratios of ethanol to buffer are increased to 1:9, 2:8, 3:7 and 4:6, maximum flux increased gradually to 69, 76, 100 and 115 μg/cm^{2}hr, respectively. The maximum flux, 115 μg/cm^{2}hr, is about 21 fold larger than the maximum flux value obtained without current. Flux maximum reached 3 to 4 hours after the current application, and after that, flux decreased with time. Figure 6(b) shows the cumulative amount delivered for 12 hours. Control study without ethanol showed 333 μg/cm^2 of cumulative transport after 12 hours. When the ratio of ethanol to buffer was increased to 1:9, 2:8, 3:7 and 4:6, cumulative amount transported increased further to 418, 511, 671 and 742 μg/cm^2, respectively. These data indicate that ethanol has better ability to enhance the transport of DH through skin than NMP. Current was off after 8 hours, and the flux through skin was investigated for 4 more hours (Figure 6(a)). Without current, flux decreased further and reached 21 μg/cm^{2}hr at 11.5 hour, when the ratio between ethanol and buffer was 4:6. For ethanol to buffer ratio of 1:9, 2:8 and 3:7, flux at 11.5 hour were 9.3, 9.4 and 17.3 μg/cm^{2}hr. These passive flux values are much larger than that observed for passive flux without any current application (Figure 3(a)). These passive flux values are also larger than those observed for passive flux after 8 hour current application with NMP treatment (Figure 4(a)). This data also indicates that ethanol has better ability than NMP in enhancing the transport of DH through skin. The bell shaped figure in flux was also observed in this transport experiment with current. As already suggested above, depletion of ethanol from the hydrogel , the decrease in concentration of DH in the donor hydrogel with time and the decrease in electroosmotic flow, due to the decreased level of

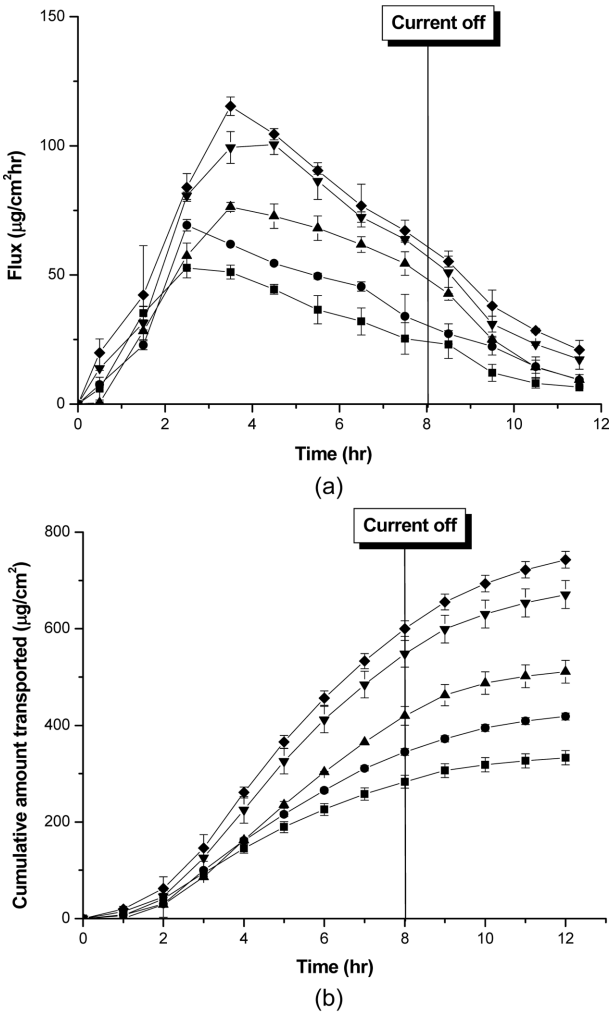

Figure 6. (a) Flux and (b) cumulative amount transported by anodal delivery across hairless mouse skin *in-vitro*. PEO hydrogel containing ethanol of different ratios (ethanol : buffer = 1:9, 2:8, 3:7 or 4:6) was used. The amount of DH in the hydrogel was 1.0 mg. Anodal current with current density of 0.4 mA/cm^2 was applied for 8 hours, and passive delivery was studied for 4 more hours. Each data point represents the mean (±S.D.) of 3 experiments. ■: 0:10 (control), ●: 1:9, ▲: 2:8, ▼: 3:7, ◆: 4:6.

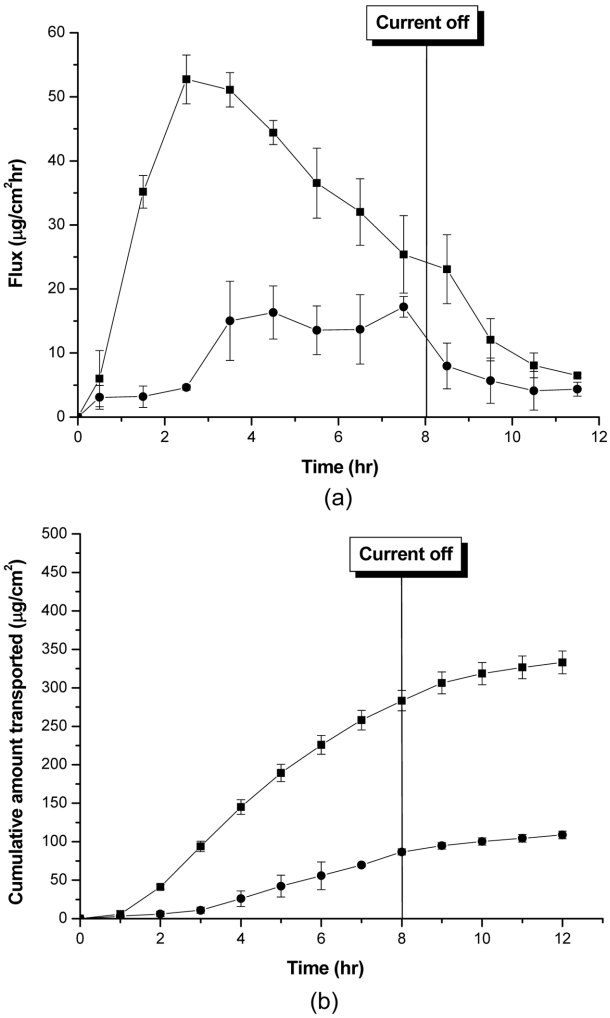

Figure 7. The effect of hydrogel pH on (a) flux and (b) cumulative amount transported by anodal delivery. Current of 0.4 mA/cm^2 was applied for 8 hours, and passive delivery was studied for 4 more hours. Each data point represents the mean (±S.D.) of 3 experiment.(at 33°C incubator). ■: pH 6.0, ●: pH 2.5.

water in the donor hydrogel could be the possible explanation for this. These data with/without current application clearly show that ethanol increases not only the passive delivery, but also the anodal iontophoretic delivery. The magnitude of increase in iontophoretic flux by ethanol also indicates that there is a large synergistic effect in flux enhancement.

Effect of pH

The flux by iontophoresis is the sum of passive diffusional flux, electro-repulsive flux and the electroosmotic flux which originates from the net negative charge of the skin at physiological pH. This net negative charge results in permselectivity to cations, and induces solvent flow from anode to cathode direction. This electroosmotic flow can enhance the transport of cationic and neutral solute molecules dissolved in the solution immersing anode. However, the transport of anions and neutral solute molecules from cathode are hindered. Permselectivity of skin and electroosmotic flow can be modulated by the change of pH of solution immersing the skin, polymeric cation like poly(L-lysine) and other positively charged molecules (Merino et a.l, 1999; Hirvonen et al., 1998; Hirvonen et al., 1997).

In this work, we have tested the effect of pH on DH transport. PEO hydrogel (100 μL) prepared using different buffer solution (pH 6.0 or 2.5) was applied on skin. Figure 7(a) shows the flux of DH at 2 different pHs. Cumulative amount trans-

ported is shown in Figure 7(b). Because donepezil (pKa 8.90) is positively charged in donor phase where pH is below 6.0, flux by electromigration should be similar magnitude in all pHs. At pH 6.0, flux reached maximum value 52.7 μg/cm^{2}hr after 3 hours, and it decreased rapidly. The main driving force for the transport at pH 6.0 seems electromigration. Electroosmosis also can contribute to the flux, but the magnitude is expected to be small (~ 4 μg/cm^{2}hr), because the electroosmotic volume flow is reported to be about 4 μg/cm^{2}hr (Oh, 2004; Sieg et al., 2004). The reason for the rapid decrease in flux is not clear. Dehydration of the hydrogel by evaporation of water might be one possible reason. Because the pH of receptor phase is 7.4, it seems highly possible that the pH of current conducting pathway of stratum corneum is increased with time. This also could be the other possible reason, but more detailed study is necessary.

At pH 2.5, flux at early time (for about 3 hours) was very small (less than 5 μg/cm^{2}hr). It is reported that the isoelectric point of current conducting pathway of skin is about 3.5-4.8 (Bath et al., 2000; Marro et al., 2001). Because the pH of the aqueous phase in hydrogel is 2.5, it seems reasonable that the pH of stratum corneum in contact with hydrogel could be changed to a value close to 2.5 at early time. At this pH, the permselectivity of skin is reported to be reversed, and the direction of electroosmotic flow is reversed. Hence the flux from anode to cathodal direction can be hindered, and low flux was observed at early time. After initial low flux, flux increased about 3 fold after 4 hours (~15 μg/cm^{2}hr). As discussed above, it seems highly possible that the pH of current conducting pathway of stratum corneum is increased with time, because the pH of receptor phase is 7.4. This will increase the pH of the current conducting pathway, and thus remove the flux hindering effect by electroosmotic volume flow. After current off, flux decreased and reached a value close to 5 μg/cm^{2}hr. This is the magnitude of passive flux, and it is about 5 fold larger than that observed without current (Figure 3(a)). This indicates that current treatment changes the skin structure, and increase the permeability.

Conclusion

In this study, we have investigated the flux enhancement of DH, using iontophoresis and chemical enhancers. We used ethanol and N-methyl pyrrolidone (NMP) as chemical enhancers in combination with iontophoresis. We also have studied the effect of pH on flux. The results show iontophoresis markedly increased the flux of DH, indicating that electromigration is playing an important role on the flux. NMP and ethanol increased not only the passive delivery, but also the anodal iontophoretic delivery. The magnitudes of increase in iontophoretic flux by these enhancers indicate that there is a large synergistic effect in flux enhancement. Flux results from pH study showed that electroosmotic flow is reversed at low pH and the flux is hindered. These results provided some information on the flux enhancing ability of ethanol and NMP in combination with iontophoresis. The data also provided some insights into the role of electromigration and electroosmosis on the flux.

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