J. Kor. Pharm. Sci., Vol. 24, No. 3, S41-S47 (1994)

Effect of Enhancers on the Electrical Properties of Skin : The Effect of Azone and Ethanol

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(Received September 3, 1994)

The effect of Azone and ethanol on the electrical properties of human and hairless mouse skin was studied and the results were compared. The complex electrical impedance was measured as a function of frequency, and resistance and capacitance were determined from Nyquist plot. After the treatment of human-heat separated epidermis with Azone, contrary to the expectation, resistance increased about 60% and it did not change with time. Capacitance also increased; immediately after the treatment, it was about 110% of pretreatment value and it increased further with time. On the other hand, when hairless mouse skin was treated with Azone, marked changes occured; resistance fell almost to the value of bathing medium itself and capacitance increased to about 200% of its pretreatment value. Similar result were obtained when hairless mouse skin was treated with 100% ethanol. The results suggest that there are differences in the strength of barrier properties of stratum corneum (SC) between human and hairless mouse skin. Overall, the results provide further mechanistic insight into ion conduction through the skin and into the role of SC lipids in skin capacitance.

Keywords—Impedance, Nyquist plot, Resistance, Capacitance, Azone, Ethanol

Introduction

Iontophoresis employs an electrical potential gradient as the driving force to enhance the permeability of charged and polar drug molecules, which are very difficult to deliver into the systemic circulation by conventional transdermal methods.[1] It provides some other advantages such as better control over the rate of drug delivery and the ease of onset and termination of delivery. With the advancement of biotechnology, iontophoresis gained particular attention due to its potential to deliver peptide and protein drugs [2-5] because these usually charged moecules are very potent but difficult to delivery by conventional methods.

Electrically, skin is usually represented as a parallel combination of capacitance and resistance.[6] It has been reported in the literature that the SC is mainly responsible for the electrical properties of the skin.[6, 7] The capacitance of the skin is believed to originate from the lipid matrix-keratin cell complex, while the resistance appears to be primarily associated with the pore pathways through the skin.[8-11] Electrical impedance (Z) measurements have shown that resistance and/or caapacitance may be affected by various factors such as hydration, ionic strength of the skin-bathing medium, pH and chemical treatment.[7, 12] The effect of iontophoresis on resis-

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tance and capacitance has also been studied.[13, 14]

In previous study,[7] the impedance of hairless mouse skin *in vitro* was measured as a function of frequency to determine how the resistance and capacitance of the membrane were affected by ionic strength, temperature and current density. Increasing the ionic strength of the bathing medium, and increasing the magnitude of current, decreased resistance, whereas capacitance was, in general, unchanged. These changes occurred rapidly. The decrease in resistance with increasing the ionic strength of the bathing medium was consistent with elevated ion levels within the ion-conducting pathways of the membrane. The decrease in resistance by increasing the magnitude of current seems to be related to the alteration of the current conducting pathway. With increasing temperature, resistance also decreased while capacitance increased.

In the present work, the effect of penetration enhancers on the electrical properties has been studied, using excised human skin and hairless mouse skin. Though iontophoresis enhances the transdermal flux, in many cases of peptide drugs, the flux is not enough to have a therapeutic effect. Iontophoresis after enhancer treatment may provide a way to solve this problem. Hence detailed understanding of the effect of enhancer on the electrical properties is very important in order to maximize the benefit from iontophoresis and also for rational design of a iontophoretic system. The enhancers studied are Azone and ethanol. Azone is one of a series of N-alkylated cyclic amides specially developed as a permeation enhancer. It is a potential enhancer for both lipophilic as well as hydrophilic drugs.[15-18] Azone seems to interact mainly with the intercellular lipids and making them more fluid, which may reduce the diffusional resistance of the penetrating drug molecules.[19] Ethanol is widely used for the permeation enhancement of drugs in many pharmaceutical products, such as creams, gels and ointments, as well as transdermal controlled release products. Though the mechanism of action is not known clearly, it is thought that osmotic swelling[20] and lipid extraction[21] are the main reason for the permeation enhancement. The effect of these enhancers on the electrical properties are hardly studied. Srinivasan *et al.*[4] employed ethanol to enhance the transdermal flux of small peptides. They observed a marked increase in leuprolide flux. However this work focused only on the flux enhancement of ethanol, not on the effect on the electrical properties.

Materials and Methods

Materials

A side-by-side, two chamber diffusion cell with 4 electrode inlets (2 for signal electrodes and 2 for sensing electrodes) was used. Each chamber held a volume of 1.9 m*l* and was magnetically stirred. All NaCl solutions were prepared with distilled water from a Milli-Q UF Plus water purification system (Millipore, Bedford, MA). Solutions were degassed under vacuum with sonication before use. The area of skin exposed to each chamber was 0.79 cm^2. Inlets in the diffusion cell permitted the positioning of signal electrodes and sensing electrodes on either side of the skin. The signal electrodes, one of which was grounded, were 1.7 cm from the epidermal and dermal surfaces; the sensing electrodes were placed at a distance of 0.4 cm. Ag/AgCl electrodes were used for their stability and reversibility. Preparation method of electrode is described in our previous work.[7]

Impedance Measurements and Data Analysis

Skin impedance measurements were made using heat separated human epidermis and full-thickness hairless mouse skin. Human skin was obtained from cadaver. Epidermis was separated by heating the skin sample in distilled water at 60℃ for 2 minutes. The epidermis samples were

stored at 4℃ in 0.1 M NaCl solution and used within 24 hours. Hairless mouse skin was obtained after sacrifice of 8~12 week old females (Simonsen, Gilroy, CA) and studied immediately.

Skin impedance was determined by the potential drop and the shift in phase (θ) across the skin measured by the lock-in amplifier using sensing electrodes. Lock-in amplifier (SR850, Stanford Research Systems, Sunnyvale, CA) measure the potential drop of alternating current of the locked-in frequency. The electric circuit employed for the impedance measurements included a 2 MΩ resistor in series with the skin. At an applied voltage of 1V (peak-to-peak), a sinusoidal current was applied via a signal generator (Hewlette Packard 8116A, Mountain View, CA) to a signal electrode positioned in one cell (the signal electrode on the other cell was grounded). Since skin impedances studied were routinely less than 150 KΩ, we could assume that the current was determined primarily by the 2 MΩ resistor in series and constant current (0.5 μA) was flowing. Because we know the potential drop across the skin and the current, we can calculate impedance of the skin from Ohm's law. The system described above was tested using equivalent RC (i.e., parallel resistor (R) and capacitor (C)) circuits, with known resistance (50 KΩ) and capacitances (58 and 98 nF). These system validation experiments revealed (a) that the assessed values of R (49.9 KΩ) and C (56 and 96 nF) were agreeing well with the component's specifications and (b) that the contribution of the instrumentation to the measured impedance was negligible.

Skin impedance was determined over a frequency range of 1~10,000 Hz. From the impedance (Z) and shift in phase (θ) measured, Nyquist plot was constructed, assuming that the skin can be represented by a simple parallel RC circuit.[6] The X and Y-axis in the Nyquist plot is called the real and imaginary part of the complex number expression of impedance, respectively. The resistance was obtained by multiplying the real part value at frequency (f_c) giving the highest imaginary part value by two. The capacitance was obtained by the equation $C=\tan\theta/(2\pi f_c R)$. The contribution of the bathing medium to impedance was ignored in the calculation because its magnitude is usually less than 1% of the impedance of skin.

Three experiments were conducted at room temperature using 0.1 M NaCl as the medium bathing both sides of the skin :

1) Experiment 1 : After mounting the heat separated epidermis in between the cells, impedance was measured. Affter dismounting the cells, skin surface was dried by blotting. About 10~15 μl of neat Azone was placed on the SC side of the skin and spread gently to cover the whole area of skin exposed to the chamber. After two hours, the surface of the skin was washed with the bathing medium and each chamber was filled with the bathing solution. The changes in impedance was followed for 16 hours.

2) Experiment 2 : Same procedure as Experiment 1 except that hairless mouse skin was used.

3) Experiment 3 : After mounting the hairless mouse skin in between the cells, impedance was measured. After dismounting the cells, skin surface was dried by blotting. Skin was wiped gently 3 times with cotton ball soaked with 100% ethanol. After this treatment skin was remounted and impedance was measured.

Results and Discussion

Effect of Azone

Fig. 1 shows the typical Nyquist plot of skin, which is generated by plotting the real component of impedance against the imaginary component for each frequency (data from Experiment 1). The corresponding equivalent electrical circuit for this semi-circular arc is a paralled RC circuit, which is shown in Fig. 1 as inlet. The intercept of the

left side of the real axis represents the solution resistance of the bathing medium (R_{sol}). The diameter of the semicircle represents the resistance of the skin (R_s). Because the resistance of the bathing solution (about 100~200 Ωcm^2) is very small compared to that of skin, the intercept of the right side can be treated as R_s. However, in this work, it was difficult to get exact value of the right side intercept (see Fig. 1), due to the difficulties in measuring the impedance at very low frequency (e.g., 0.01 Hz). Hence the resistance was determined by multiplying the real part value at critical frequency (f_c) giving the highest imaginary part value by two, assuming that the semicircle is symmetrical.

Fig. 1 shows that after the Azone treatment, resistance increased. The change in resistance with time after treatment is shown in Fig. 2. The resistance increased about 60% of its pretreatment value. In general, the resistance stayed unchanged with time. This result is quite surprising, because usually the enhancers are known to disrupt the lipid matrix in the SC and thus may create new pathways for polar and charged molecules which are otherwise cannot penetrate. This new pathways may also serve as the pathway for current and decrease the electrical resistance. Konturri *et al.*[23] also observed that resistance increases after Azone treatment. It is not clear why resistance increases. Literature strongly suggest that current flow occurs mainly through pores present at appendages, such as hair follicles and sweat glands.[8-10] A plausible explanation might be the blockage of these pores by Azone. Fig. 3 shows the change of capacitance with time after Azone treatment. Capacitance increased after Azone treatment and it further increased with

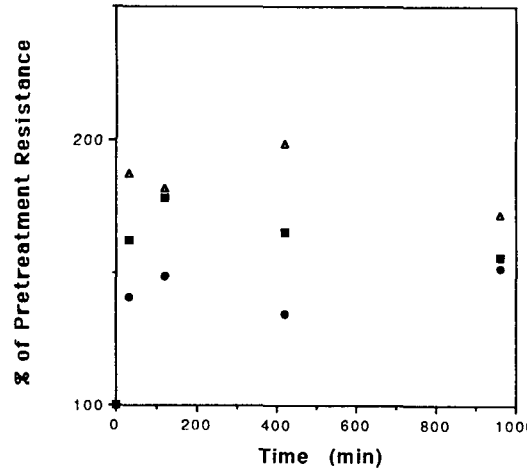

Figure 2—The change in resistance with time after the Azone treatment of human epidermis.

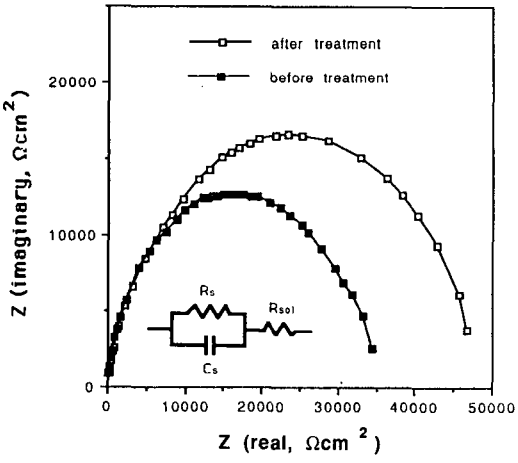

Figure 1—Typical Nyquist plot of human epidermis before and after Azone treatment. Corresponding equivalent electrical circuit is shown as inlet.

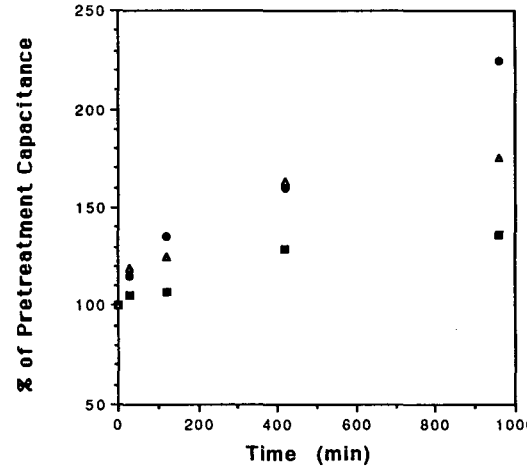

Figure 3—The change in capacitance with time after the Azone treatment of human epidermis.

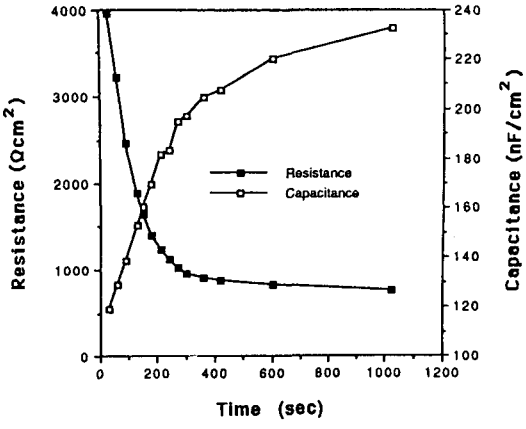

Figure 4—The change in resistance and capacitance with time immediately after the Azone treatment of hairless mouse skin

Table I—*The Change in Resistance and Capacitance of Hairless Mouse Skin before and after the Treatment with Azone*

Treatment	Resistance(kΩ cm²)	Capacitance(nF/cm²)
before	98.2 ± 29.5	55 ± 15
after*	2.1 ± 0.5	191 ± 53

* measured at 15 minutes after the skin was in contact with the bathing medium after the Azone treatment.

time. Azone is an extremely lipophilic material.[15] It dissolves readily in the skin lipids; it is likely to associate with the lipids and it is unlikely to partition significantly out of skin. This interaction induces the disordering of the lipids in SC lipid matrix[23], which in turn increase the ability of these lipid molecules to orient themselves to the electric field. Azone does not penetrate into corneocytes[24]. Azone's extreme lipophilicity might also be the reason why the increased resistance stayed nearly unchanged for 16 hours; once Azone blocks the current flowing pores, it would not partition out to the bathing medium.

Fig. 4 shows a typical results obtained after the treatment of hairless mouse skin with Azone. The resistance and capacitance of skin before treatment were 105,000 Ω cm² and 72 nF/cm², respectively. After treatment resistance dropped markedly to 4,000 Ω cm² and it decreased further with time. Capacitance, on the other hand, increased after the treatment and it increased further with time. The changes in resistance and capacitance are summarized in Table I. Resistance decreased to 2% of its pretreatment value and capacitance increased 3.5 fold. This results indicate that hairless mouse skin is more vulnerable to the penetration of Azone than human skin. The decrease in resistance is expected to be due to the conformational disorder of the lipid alkyl chains caused by Azone, which might create new 'defects' in the lipid matrix of SC, through which ions can now pass more freely. The large conformational disorder of the lipid alkyl chains may also results in a considerable enhancement of the skin's dielectric constant (permittivity) and, in consequence, a substantial rise in capacitance.

Effect of Ethanol

Ethanol is known to increase the permeability by both delipidization and osmotic swelling of the skin.[20, 21] Based on the permeability data of estradiol which deviated significantly from the classical lipid barrier model, Ghanem *et al.*[20] suggested that highly concentrated ethanol may induce new or activated latent pores in the SC. Scheuplein *et al.*[25] showed that when neat primary alcohols were placed on the SC side only, they might cause irreversible damage to the skin. The work of Bommannan *et al.*[21] shows that ethanol does not disorder SC lipid domains and relatively short-term exposure causes significant lipid extraction.

Table II shows the results of ethanol treatment on hairless mouse skin. After treatment, resistance decreased to 9% of pretreatment value and the capacitance increased 1.7 fold. This result is similar to that observed for Azone treatment on hairless mouse skin. One thing interesting is the recovery of the resistance with time. Though data is not shown, we have observed slow but continuous increase in resistance upto 4 hours. The decrease in resistance is thought to be due to the

Table II—*The Change in Resistance and Capacitance of Hairless Mouse Skin before and after the Treatment with Ethanol*

Treatment	Resistance($k\Omega\,cm^2$)	Capacitance(nF/cm^2)
before	72.4 ± 17.1	61 ± 16
after, immediately	6.2 ± 1.5	101 ± 9
after, 1 hour later	9.3 ± 3.8	103 ± 23

new or activated latent pores in the SC as suggested by Ghanem *et al.*,[20] which provide new pathways for the flow of current and thus decrease the resistance. It is probably created by 1) osmotic swelling of the lipid domain by ethanol and 2) lipid extraction. These pores probably is filled with water. This also increases the dielectric constant of the SC lipid domain and thus increase the capacitance. The pores or defects created by Azone or ethanol are probably different in nature, because Azone is known to increase the fluidity (disordering) of the SC lipids, but ethanol increases the orderness of the SC lipids. However the net results are similar; decrease the resistance and increase the capacitance. The reason why the resistance recovers slightly with time is not clear. One possible speculation is that the ethanol molecules which penetrated into the lipid domain are diffusing out of the skin to the bathing medium. This decreases the volume of lipid domain and thus increase the resistance.

The result obtained in this work agree well with the flux data of insulin analogues by iontophoresis.[26] With gentle wiping with ethanol, they increased the flux 1000 fold. The results of Srinivasan *et al.*[4] also show good agreement.

Conclusions

The effect of Azone and ethanol on the electrical properties were studied using human epidermis and hairless mouse skin. Both Azone and ethanol decreased the resistance and increased the capacitance of hairless mouse skin, though the mechanisms by which these results are generated seem to be different with each other. In human epidermis, after Azone treatment, both resistance and capacitance increased. The increase in resistance seems to be related to the blockage of the current flowing pores. The results suggest that human skin has stronger barrier properties than hairless mouse skin. Overall, the results provide further mechanistic insight into ion conduction through the skin and into the role of SC lipids in skin capacitance.

References

1) R.R. Burnette, Iontophoresis, In Transdermal Drug Delivery, J. Hadgraft & R. Guy (Eds.), Marcel Dekker Inc., New York, 247-291 (1989).
2) P.G. Green, R.S. Hinz, A. Kim, C. Cullander, G. Yamane, F.C. Szoka, Jr. and R.H. Guy, Transdermal iontophoresis of amino acids and peptides *in vitro*, *J. Controlled Release*, **21**, 187-190 (1992).
3) R.R. Burnette and D. Marrero, Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin, *J. Pharm. Sci.* **75**, 738-743 (1986).
4) V. Srinivasan, M. Su, W.I. Higuchi and C.R. Behl, Iontophoresis of polypeptides : Effect of ethanol pretreatment of human skin, *J. Pharm. Sci.*, **79**, 588-591 (1990).
5) C. Cullander and R.H. Guy, Transdermal delivery of peptides and proteins, *Advanced Drug Delivery Reviews*, **8**, 291-329 (1992).
6) T. Yamamoto and Y. Yamamoto, Electrical properties of the epidermal stratum corneum, *Med. Biol. Eng. March*, 151-158 (1976).
7) S.Y. Oh, L. Leung, D. Bommannan, R.H. Guy and R.O. Potts, Effect of current, ionic strength and temperature on the electrical properties of skin, *J. Controlled Release*, **27**, 115-125 (1993).
8) C. Cullander, What are the pathways of iontophoretic current flow through mammalian

skin?, *Advanced Drug Delivery Reviews*, **9**, 119-135 (1992).
9) C. Cullander and R.H. Guy, Sites of iontophoretic current flow into the skin : Identification and characterization with the vibrating probe electrode, *J. Invest. Dermatol.*, **97**, 55-64 (1991).
10) R.R. Burnette and B. Ongpipattanakul, Characterization of the pore transport properties and tissue alteration of excised human skin during iontophoresis, *J. Pharm. Sci.*, **76**, 765-773 (1987).
11) S.Y. Oh, R.A. Siegel and R.H. Guy, Mathematical modelling of the resistance of skin, *in preparation.*
12) A.C. Allenby, J. Fletcher, C. Schock and T.F.S. Tees, The effect of heat, pH and organic solvents on the electrical impedance and permeability of excised human skin, *Br. J. Derm.*, **81**, 31-39 (1969).
13) D. Foley, J. Corish and O.I. Corrigan, The use of complex impedance to examine the effect of passive and iontophoretic transdermal drug transport through excised human epidermal tissue, *Proceed. Intern. Symp. Control. Rel. Bioact. Mater.*, **17**, 427-428 (1990).
14) R.R. Burnette and T.M. Bagniefski, Influence of constant current iontophoresis on the impedance and passive Na^+ permeability of excised mouse skin, *J. Pharm. Sci.*, 77, 492-497 (1988).
15) R. Vaidyanathan, V.J. Rajadhyaksha, B.K. Kim and J.J. Anisko, In Transdermal delivery of Drugs, A.F. Kydonieus and B. Berner (Eds.), CRC Press, Boca Raton, FL, chapter 5 (1987).
16) R.B. Stoughton and W.O. McClure, Azone enhances percutaneous absorption, Presented at the 41st Annual Meeting of American Academy of Dermatology, New Orleans, LA, Dec. 4-9 (1982).
17) J.W. Wiechers and R.A. DeZeeuw, Transdermal drug delivery : Efficacy and potential applications of the penetration enhancer Azone, *Drug Discovery Delivery* **6**, 87 (1990)
18) B.W. Barry, Effect of penetration enhancers on the permeation of mannitol, hydrocortisone and progesterone through human skin, *J. Pharm. Pharmacol.* **39**, 535 (1987).
19) J.C. Beastal, J. Hadgraft and C. Washington, Mechanism of action of Azone as a percutaneous penetration enhancer : Lipid bilayer fluidity and transition temperature effects, *Int. J. Pharm.*, **43**, 207-213 (1988).
20) A.H. Ghanem, H. Mahmoud, W.I. Higuchi, U. D. Rohr, S. Borsadia, P. Liu, J. Fox and W. Good, The effects of ethanol on the transport of β-estradiol and other permeants in hairless mouse skin II. A New quantitative approach, *J. Controlled Release,* **6**, 75-83 (1987).
21) D. Bommannan, R.O. Potts and R.H. Guy, Examination of the effect of ethanol on human stratum corneum using infrared spectroscopy, *J. Controlled Release,* **16**, 299-304 (1991).
22) K. Kontturi, L. Murtomäki, J. Hirvonen, P. Paronen and A. Urtti, Electrochemical characterization of human skin by impedance spectroscopy : The effect of penetration enhancers, *Pharm. Res.*, **10**(3), 381-385 (1993).
23) J. Hirvonen, R. Rajala, E. Laine, P. Paronen, and A. Urtti, Penetration enhancers dodecyl N, N-dimethylamino acetate and Azone alter the structure of the skin-A DSC study. In R.C. Scott, H.E. Dodde, R.H. Guy, and J. Hadgraft (eds.), Proceedings of the 2nd Conference of Prediction of Percutaneous Penetration, *Southampton* **2**, 350-354 (1991).
24) M. Goodman and B.W. Barry, Differential scanning calorimetry (DSC) of human stratum corneum : Effect of Azone, *J. Pharm. Pharmacol.*, **37**(suppl.), 1-80 (1985).
25) R.S. Scheuplein and I.H. Blank, Mechanism of percutaneous absorption. IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids, *J. Invest. Dermatol.*, **60**(5), 286-296 (1973).
26) L. Langkjær, J. Brange, G.M. Grodsky and R. H. Guy, Transdermal delivery of monomeric insulin analogues by iontophoresis, *Proceed. Inter. Symp. Control. Rel. Bioact. Mater.*, **21**, 172-173 (1994).