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올레인산 및 프로필렌글리콜이 피부의 전기적 성질에 미치는 영향

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The Effect of Oleic Acid and Propylene Glycol on the Electrical Properties of Skin

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The effects of oleic acid, propylene glycol and 5% (w/w) oleic acid in propylene glycol on the electrical properties of hairless mouse skin were studied and the results were compared. The complex electrical impedance was measured as a function of frequency, and resistance and capacitance were determined from the Nyquist plot. Immediately after the treatment with oleic acid, resistance was 145% of the pretreatment value. However it decreased with time and, after 20 hours, it was about 25% of its pretreatment value. Capacitance increased; immediately after the treatment, it was about 125% of pretreatment value and it seemed to increase slowly with time. When the skin was treated with propylene glycol, resistance decreased about 50% and capacitance increased about 65%. Similar results were observed when the skin was treated with 5% (w/w) oleic acid in propylene glycol, except that the magnitude of resistance drop was much larger. Oleic acid acted synergistically with propylene glycol. Together with the flux data in the literature, the results obtained in this work indicate that electrical resistance is closely related to the permeability of drug molecules through the skin. The results are discussed in terms of the mechanism of action of these penetration enhancers. Overall, this work provided further mechanistic insight into the role of SC lipids in skin resistance and capacitance.

Keywords—Impedance, Nyquist plot, Resistance, Capacitance, Oleic acid, Propylene glycol, Synergistic effect

Electrically, skin is usually represented as a parallel combination of capacitance and resistance.[1] From the stripping experiments, it has been shown that the stratum corneum (SC) is mainly responsible for the electrical properties of the skin.[1,2] The capacitance of the skin is thought to originate from the lipid matrix-keratin cell complex, while the resistance appears to be primarily associated with the current flowing pores.[3-6] These pores are mainly locating at appendages on skin, such as hair follicles and sweat glands, though there are some unidentified pathways whose contribution to the flow of current is dependent on the magnitude of current.[4,7] Electrical impedance (Z) measurements have shown that resistance and/or capacitance may be affected by

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various factors such as hydration, ionic strength of the skin-bathing medium, pH and chemical treatment.[2,8] The effect of iontophoresis on resistance and capacitance has also been studied.[9,10]

In our previous work,[11] we have studied the effect of Azone and ethanol on the electrical properties of skin. Both Azone and ethanol decreased the resistance and increased the capacitance of hairless mouse skin. It seemed that the mechanisms by which these results are generated are different with each other. In human epidermis, after Azone treatment, both resistance and capacitance increased. It was speculated that the increase in resistance was due to the blockage of the current flowing pores through the skin by Azone. The results suggested that human skin has stronger barrier properties than hairless mouse skin.

In this work, we further investigated the effect of penetration enhancers on the electrical properties of skin, using hairless mouse skin. The enhancers studied are oleic acid (OA), propylene glycol (PG) and 5% (w/w) OA in PG. The effects of these enhancers on the electrical properties are hardly studied. OA is an unsaturated fatty acid with 18 carbon atoms. It has been known to be effective enhancers for polar and moderately polar drug molecules. Based on the DSC (Differential Scanning Calorimetry) and FTIR (Fourier Transform Infrared Spectroscopy) studies, it has been postulated that OA exists in a separate phase as liquid in the SC and forms a number of permeable defects at the liquid-solid (SC lipids) interfaces.[12,13] PG is a commonly used solvent in many topical formulations, due to its good solvent properties for both hydrophilic and lipophilic compounds.[14-16] It has been shown to have direct effect on skin barrier function.[17] It has been used as a cosolvent for other penetration enhancers, such as Azone, terpenes and oleyl surfactants.[18-20] The mode of action of PG is not clear, though there are some suggestions that it produces some change in the hydration layer in the lipid bilayer.[21]

EXPERIMENTAL

Cells and Electrodes

A side-by-side 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. The area of skin exposed to each chamber was 0.785 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 from the surfaces. Ag/AgCl electrodes, which were prepared electrochemically, were used due to their stability and reversibility. A short length (3 mm) of Ag wire (99.9%, Aldrich, Milwaukee, WI) (1 mm diameter) was lightly sanded with emery paper, washed in acetone, and then cleaned in 1 M HCl for 20 minutes at 50℃. After rinsing with distilled water, the Ag was anodically plated with AgCl (using a Pt cathode) by immersion in 0.5M KCl and application of a 0.1 mA current. Sensing electrodes were plated for 20 minutes, signal electrodes for 5 hours.

Impedance Measurements and Data Analysis

Skin impedance measurements were made using excised full-thickness hairless mouse skin of 8-12 week old females (Simonsen, Gilroy, CA) obtained immediately after sacrifice. Skin impedance was determined by the potential drop and the shift in phase (θ) across the skin measured by a lock-in amplifier (SR530, Stanford Research Systems, Sunnyvale, CA) using sensing electrodes. The electric circuit employed for the impedance measurements included a 2 MΩ resistor in series with the skin. A sinusoidal current (1V peak-to-peak) 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 150KΩ we could assume that the current was determined primarily by the 2 MΩ resistor in series and constant current (0.5 μA) was flowing. Impedance of the skin was calculated by Ohm's law.

Skin impedance was determined over a frequency range of 1−5.000 Hz. From the impedance (Z) and shift in phase (θ) measured at each frequency, Nyquist plot was constructed. The X and Y-axis in the Nyquist plot are 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 (fc) giving the highest imaginary part value by two. The capacitance was calculated by the equation $C=\tan\theta/(2\pi fcR)$. 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 :

(i) Experiment 1 : The resistance and capacitance of the skin were determined before OA treatment. After dismounting the cells, skin surface was dried by blotting. About 10−15 μl of neat OA was placed on the SC side of the skin and spread gently to cover the whole area of skin exposed to the chamber. After one hour, the surface of the skin was washed 3 times with 0.1 M NaCl solution. The changes in impedance were followed for 20 hours.

(ii) Experiment 2 : Same procedure as Experiment 1 except that PG was used for skin treatment in stead of OA.

(iii) Experiment 3 : Same procedure as Experiment 1 and 2 except that 5% (w/w) OA in PG was used for skin treatment.

RESULTS AND DISCUSSION

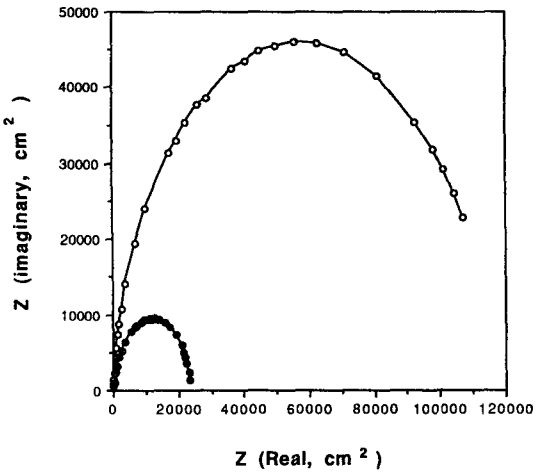

Figure 1−Nyquist plot of hairless mouse skin before (○) and after (●) oleic acid treatment.

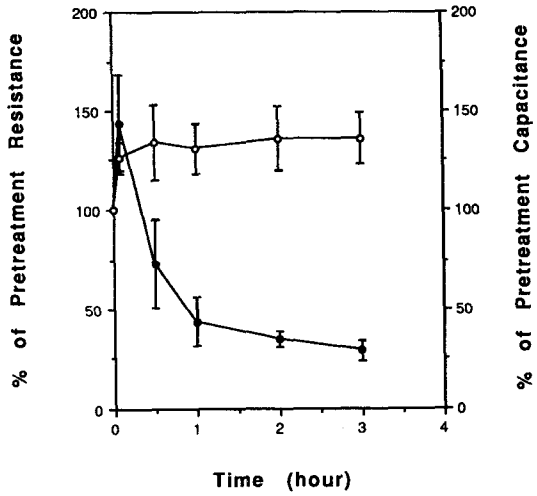

Figure 2−The change in resistance (●) and capacitance (○) with time, after the skin is treated with oleic acid. The value at time zero (100%) indicates the pretreatment value (n=5).

Effect of Oleic Acid

The average resistance and capacitance of hairless mouse skin used in this work are 129 ± 40 $K\Omega cm^2$ and 50 ± 12 nF/cm^2, respectively. Fig. 1 shows the Nyquist plot of skin before and after the treatment with OA. The data obtained at 20 hours after treatment is used for the plot. The semi-circular shape indicates that, electrically, the skin can be treated as a parallel RC circuit. The

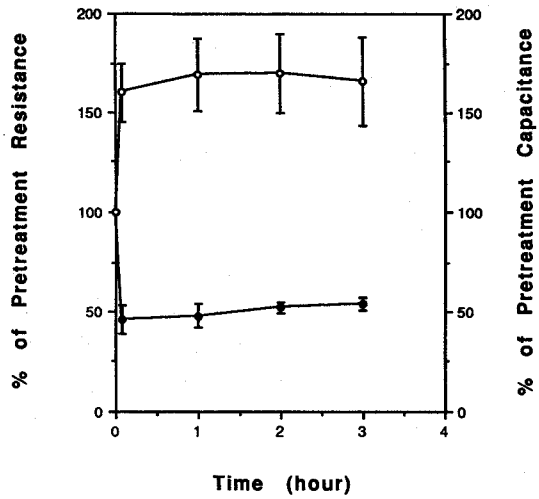

Figure 3—The change in resistance (●) and capacitance (○) with time, after the skin is treated with propylene glycol. The value at time zero (100%) indicates the pret reatment value (n=5).

diameter of the semi-circle shows that there is a large decrease in resistance. The change in resistance and capacitance with time, after the skin is treated with OA, is shown in Fig. 2. Immediately after the treatment with OA, resistance was about 45% larger than pretreatment value. However it decreased with time and, after 20 hours, it was about 25% of its pretreatment value. On the other hand, capacitance increased to 125% of its pretreatment value immediately after the treatment and it seemed to increase slowly with time.

Using FTIR and per-deuterated OA, Ongpipattanakul *et al.*[12] was able to monitor the phase behavior of exogenously added OA and endogenous SC lipids independently. They observed that added OA decreased the lipid phase transition temperature in addition to increasing the conformational disorderness of the endogenous lipid alkyl chains above their phase transition temperature. At temperatures lower than the lipid phase transition, the orderness of SC lipids was not affected by the incorporation of OA. They also found that OA, itself, was almost fully disordered at temperatures both above and below the endogenous lipid phase transition temperature in the intact SC and extracted lipid samples. These findings suggest that OA exists as a liquid within the ordered SC lipids. The results strongly support the proposal that OA exists as a separate phase in the SC lipids and forms a number of permeable defects at the interface between the SC lipids and OA.[13] Furthermore, there are indications that these defects are associated with water.[22] The decrease in resistance after OA treatment is probably due to these permeable defects which have water molecules associated with them. Current can flow through these defects rather easily than through the SC lipids which is in highly ordered state. The increase in resistance immediately after treatment seems to be related to the blockage of the current conducting pathways at the appendages or the coating of skin surface by remaining OA after washing. The decreases in resistance with time might be explained by the gradual washout of these OA from the skin surface or from the pores at the appendages. The increase in capacitance may also be related to the micro domains of OA; the dielectric constant of OA domain is probably much larger than SC lipid domain due to the water molecules around them, thus in average increase the dielectric constant of the SC lipid.

Effect of Propylene Glycol

The change in resistance and capacitance with time, after the skin is treated with PG, is shown in Fig. 3. Resistance decreased about 50% and capacitance increased about 65%. Though not shown in Fig. 3, capacitance decreased slowly with time and after 20 hours it was about 140% of pretreatment value. Resistance, on the other hand, increased slightly with time.

The effect of PG as a penetration enhancer is not clear. By use of NMR spectroscopy and deuterated water in nonionic surfactant bilayers (n-dodecyltetraoxyethylene glycol ether in water) as a model system for SC, it was observed that PG

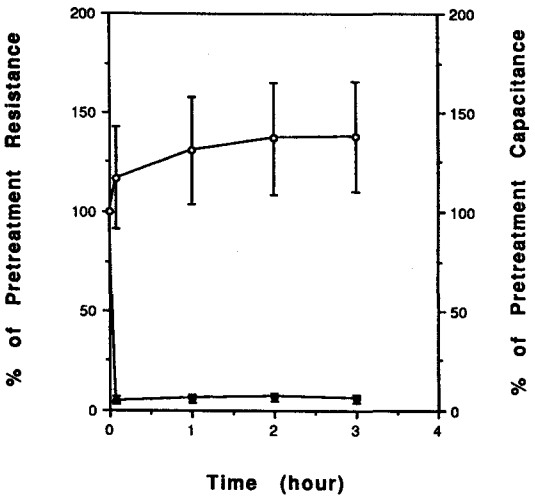

Figure 4—The change in resistance (●) and capacitance (○) with time, after the skin is treated with 5% (w/w) OA in PG. The value at time zero (100%) indicates the pretreatment value (n=5).

competes with water for the hydration sites and the redistribution of water molecules occurs.[21] A semiquantitative estimates of the amount displaced indicates that 2−3 moles of water per mole of surfactant are replaced by 1 mol of PG. Similar results were obtained in x-ray diffraction measurement, showing the competition for solvation sites of the polar head group of the surfactant.[21] The incorporation of PG in this polar head group region may disrupt the packing of surfactants at this region, where the packing is most sensitive, and decrease the orderness of the bilayer. Thus the barrier property of the bilayer may be reduced.

It seems that this disruption of the lipid orderness in SC lowers the resistance for the flow of ions. In our previous work,[2] we have found that there is a dramatic change in the resistance and capacitance at the gel-to-liquid crystalline phase transition of SC lipids. Bramhall[23] also observed large decrease in resistance at pase transition temperature. These results indicate that as disorderness of lipids in SC increases, resistance decreases. Increase in dielectric constant, which is probably caused by the increased partition of water molecules into the disordered lipid domain, may also contribute to the decrease in resistance.[24]

The decrease in resistance is smaller than the OA treated skin, where it decreased to about 25% of pretreatment value. This result is consistent with the nitroglycerin flux data, which showed that flux was much larger when the skin was treated with OA.[25] Slight increase in resistance is probably related to the slow redistribution of PG into the bathing medium, thus making the lipid domain more ordered. The increase in capacitance is probably due to the increased dielectric constant of the lipid domain. Better orientation of the lipids in SC to the applied electric field might also contribute to the increase.

Effect of 5% Oleic Acid in Propylene Glycol

The change in resistance and capacitance with time, after the skin is treated with 5% (w/w) OA in PG, is shown in Fig. 4. Resistance decreased markedly to about 5 % and capacitance increased to about 135% of its pretreatment value. These values did not change with time. The magnitude of increase of capacitance is similar to that obtained after either neat OA treatment or PG treatment. However the drop in resistance is much larger. Loftsson *et al.*[26] showed that mixtures of PG with OA exhibited synergism in the permeability of drugs. Synergistic effect was most pronounced for polar drugs. For example, when the vehicle contained 2% (v/v) OA, the permeability of acyclovir from a saturated solution in PG increased more than a hundred fold. On the other hand, the increase for estradiol from the same vehicle was only 1.3 fold. This synergistic effect of 5% (w/w) OA in PG system is clearly reflected as a marked drop in the electrical resistance of skin, as shown in Fig. 4. One possible explanation for this synergistic effect is the facilitated incorporation of OA into the SC lipid alkyl domain by the interaction of PG at the polar head group

region.

CONCLUSIONS

The effect of OA, PG and 5% (w/w) OA in PG on the electrical properties of hairless mouse skin were studied. In all cases, resistance decreased and capacitance increased, though the mechanisms by which these results are generated seem to be different with each other. Treatment with 5 % (w/w) OA in PG showed synergistic effect in decreasing the resistance of skin. The results obtained in this work, together with the flux data in the literature, indicate that electrical resistance is closely related to the permeability of drug molecules through the skin. Overall, this work provided further mechanistic insight into the role of SC lipids in skin resistance and capacitance. The increase in capacitance is probably due to the combined effect of each enhancers; increase in dielectric constant and fluidity of the SC lipid.

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