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Interaction of percutaneous absorption enhancer with stratum corneum of hamster cheek pouch; an electrophysiological study

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Summary

The effects of 1-dodecylazacycloheptan-2-one (Azone) and sodium lauryl sulfate (SLS) on the permeability to drugs of stratum corneum sheets (SC) isolated from hamster cheek pouch were investigated in vitro. By using an electrophysiological technique, the electrical resistance (R_m) of the SC was determined by measuring its permeability to various drugs. It was shown that the isolated SC behaved as a lipid barrier to drug permeation and possessed high resistivity to ion flow. Pretreatment of the SC with SLS caused a marked decrease in R_m, indicating that its effect on drug permeability depends on its capacity to reduce the barrier function of the SC. On the other hand, Azone enhanced mainly permeability to lipophilic drugs and did not result in significant change in R_m. It was suggested that Azone promoted drug permeation across the SC without reducing its role as a barrier to hydrophilic substances. The electrophysiological technique used in this study was believed to be useful for examining the interaction of some percutaneous absorption enhancers with SC.

Introduction

Owing to the highly resistant barrier of the skin, the use of a number of adjuvants, which temporarily diminish impermeability to drugs, is often found to be necessary in order to achieve drug delivery into the body via topical application. In recent years, many chemicals have been reported to have the ability to promote drug absorption from the skin (Walters, 1988; Barry, 1989; Catz and Friend, 1989). The major barrier to percutaneous absorption is accepted as being the stratum corneum (SC), the outer keratinized thin layer of the skin. Therefore, it may readily be deduced that such adjuvants (designated as percutaneous absorption enhancer) interact primarily with the SC and promote the drug penetration through it. However, direct investigation of the interaction of enhancers with the SC is prevented

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by the presence of hair follicles or sweat ducts. The barrier function is lost in isolated SC, since holes are created at the sites of such appendages after isolation. Morimoto et al. (1986) demonstrated that 1-dodecylazacycloheptan-2-one (Azone) mainly affects the permeability to drugs of the SC, and not that of other skin layers, since its enhancing effect on stripped skin is much smaller than that on intact skin. Consequently, previous studies on the effects of adjuvants on the SC often involved estimation of this factor by an indirect method.

In this study, we isolated intact SC from hamster cheek pouch which does not include any appendages, and the effects of Azone and sodium lauryl sulfate (SLS) on its permeability to drugs were directly evaluated under in vitro conditions. Further, from the change in the electrical resistance of the SC, the effects of both enhancers on the barrier functions of the SC were also examined.

Materials and Methods

Materials

Azone was supplied by Nelson Research (Irvine, CA) and was used as received. Trypsin (purified porcine pancreas, type IX) was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were reagent grade products obtained commercially.

Preparation of isolated SC membranes

Isolated sheets of the SC were prepared according to the previously described method (Kurosaki et al., 1989). Briefly, excised cheek pouch from a male golden hamster (120–160 g body weight) was spread out, and its lamina propria side was incubated for 4 h at 37°C on filter paper with trypsin solution (0.5%, pH 8.0). Thereafter, the SC was peeled away from the underlying layers and immediately mounted in an Ussing-type chamber (Yamashita et al., 1986). Both sides of the membrane were filled with Ringer solution (11 ml per side) and bubbled with air to stir the solution. The composition (mM) of the standard Ringer solution was 150 NaCl, 0.38 KH_2PO_4, 2.31 K_2HPO_4 and 0.25 $CaCl_2$ (pH 7.4). The cross-sectional area of the chamber was 0.75 cm^2 and the entire chamber was placed in a temperature-controlled box (at 37°C) throughout the experiment.

Measurement of the electrical resistance of the SC

Electrical resistance (R_m) of the SC was calculated from the change in transmembrane potential difference (ΔPD) caused by the flow of a constant external current across the membrane. The external current was passed via the Ag/AgCl electrodes placed at each edge of the chamber. The ΔPD resulting from the external current was measured between two salt bridges (150 mM NaCl in 3% agar) connected via calomel electrodes to measuring equipment (Yamashita et al., 1986). The ΔPD signals were amplified by a differential amplifier (using FET-operational amplifier, Model 1026, Teledyne Philbrick, U.S.A.) and measured on a digital multimeter (9100EA, Sanwa Co., Tokyo, Japan). The R_m of the SC was evaluated according to Ohm's law from the linear portion of the curve shown in Fig. 1, which represents the relationship between the external current and the ΔPD (see Results). The resistance of the Ringer solution present between the tips of the two salt bridges is below 10 Ω cm^2 (less than 1% of R_m of SC), and thus its contribution to the total resistance was considered to be negligible in all experiments.

Pretreatment of SC with Azone or SLS

The SC in the chamber was equilibrated with standard Ringer solution for 30 min, after which Ringer solution containing the appropriate concentration of Azone or SLS was introduced into the luminal side (donor side) of the SC. Ringer solutions containing Azone were prepared by emulsifying the required concentration of Azone with 0.1% polysorbate 20 (Morimoto et al., 1986). SLS was dissolved directly. The SLS-containing Ringer solution was prepared to be identical in both osmolarity and sodium concentration with the standard Ringer solution by reducing the concentration of NaCl. The pretreatment was continued for 1 h. In the control experiment, the

SC was pretreated using standard Ringer solution in the same way.

Measurement of drug permeation through SC

After pretreatment, both sides of the SC were washed and Ringer solution containing the drug under test was introduced into the donor side, standard Ringer solution being added to the other side (receptor side). Permeability measurements were carried out on the following drugs: methyl paraben (MP), theophylline (THP), 5-fluorouracil (5FU) and sulfanilic acid (SA). Initial drug concentrations on the donor side were 1 mM (MP), 10 mM (both THP and 5FU) and 20 mM (SA). In each experiment, the osmolarity of the Ringer solution on the receptor side was adjusted to that on the donor side by addition of an appropriate concentration of mannitol. Aliquots were removed at appropriate intervals (every 30 min or 1 h) from the receptor side up to 3 h for MP and 5 h for the other drugs. The volume of the solution was kept constant by replenishment with fresh Ringer solution.

Analytical method

MP, THP and 5FU were determined according to a high-performance liquid chromatographic (HPLC) method. A high performance liquid chromatograph (LC-6A, Shimadzu Co., Kyoto, Japan) equipped with a variable-wavelength UV detector (SPD-6A, Shimadzu) was used with a reversed-phase column (Inertsil ODS-2, Gasukurokogyo, Tokyo, Japan). The analytical conditions for each drug have been described previously (Kurosaki et al., 1986; Kadir et al., 1989; Yamashita et al., 1989).

SA was determined spectrophotometrically as described by Kimura et al. (1981).

Results

Electrical feature of SC

Fig. 1 shows the relationship between the external constant current and ΔPD across the SC. A linear relationship was observed at current strengths below 5 μA (corresponding to a current density of 6.67 $\mu A/cm^2$), which changed into a nonlinear form as the strength of the current exceeded 20–50 μA. Tregar (1966) demonstrated that the induction of a high transmembrane potential difference (> 1–2 V) by a strong electrical current across the human epidermis results in electrical damage to the SC and in non-Ohmic behavior. He suggested that the electrical resistance of the skin should be measured using a weak electrical current. In our case, ΔPD greater than about 500 mV also damaged the SC and, in such ranges, the apparent R_m value calculated via Ohm's law (ΔPD/current density) decreased. The lower R_m of the SC due to the constant current (100 μA, for 30 min) did not return to its initial value (Fig. 2), suggesting that irreversible damage was caused to the SC. Therefore, in subsequent experiments, ΔPD was measured using only 0–3 μA current strength and the R_m of the SC was calculated from the slope of the linear regression line.

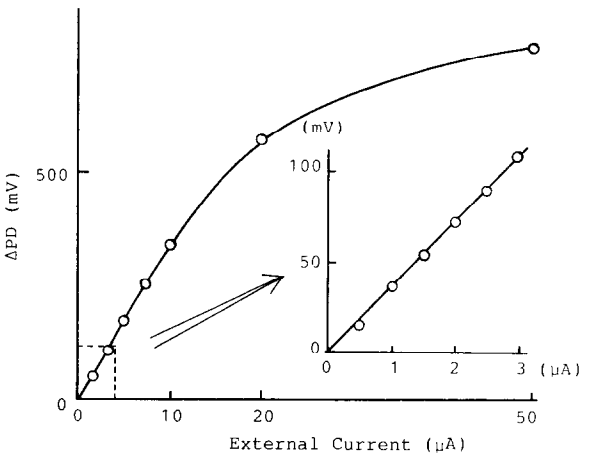

Fig. 1. Relationship between external constant current and ΔPD across the SC. (Inset) Linear relationship as observed for current strengths below 3 μA.

Another problem in measuring R_m is that of membrane polarization caused by the passage of an electrical current across the membrane (Tregar, 1966). Dugard et al. (1973) measured the impedance of human epidermis using alternating current (a.c.) of low frequency, in which membrane polarization could be avoided, since charge accumulation around the membrane was excluded. They suggested that, at low frequencies,

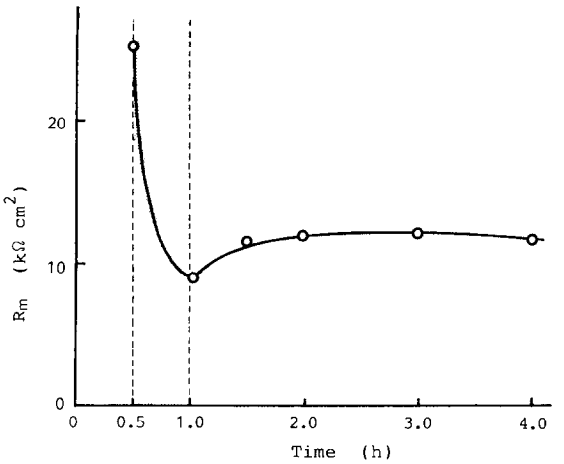

Fig. 2. Effect of constant current on R_m of the SC. A constant current (100 μA) was passed across the SC for 30 min (indicated by stippled area).

the impedance of the membrane could be regarded as almost equal to its R_m. We also measured the impedance of the SC of hamster cheek pouch by using an impedance analyzer (Hewlett Packard 4194A) and, from the dependency on the frequency of the a.c. current, calculated the R_m of the SC. The directly measured values of R_m using a constant current correlated very closely with those calculated, and differences in the absolute values were only 10–20% in each SC (data not shown). Since the main purpose of our experiments was to compare the change in R_m with the permeability of SC to drugs after pretreatment with adjuvants, we felt justified in adopting the simple method for measuring R_m, in which a weak constant current is employed.

Under control conditions, the R_m values of the SC were in the range of 20–40 kΩ cm^2 and declined gradually after the SC had been mounted in the chamber (Fig. 3). In addition, the R_m of isolated SC was about 80% of the value for intact tissue of the cheek pouch. These R_m values are extremely high when compared with those of other biomembranes (Powell, 1981), and comparable to those reported by Evans et al. (1979) and Reid et al. (1986) for intact tissues of hamster cheek pouch.

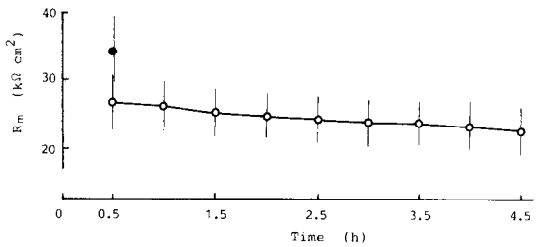

Fig. 3. Time course of R_m of the SC after mounting of the SC in the chamber. (○) R_m of the isolated SC; (●) (at 0.5 h) R_m of intact tissue of hamster cheek pouch. Each point represents the mean ± S.E. of at least three experiments.

Drug permeation through SC

The time course of drug permeation through the SC is demonstrated in Fig. 4. Since the permeation of all the drugs, except SA, varied linearly with time after the lag-time of 30–60 min, drug flux rates were estimated from the slope of the linear portion. Further, the permeability coefficient (K_p) of the drug with respect to the SC was evaluated by dividing the flux rate by the drug concentration on the donor side, assuming that the latter factor remained constant and sufficiently greater than that on the receptor side throughout the experiment. This assumption would appear to be acceptable since even in the case of MP, the most permeable drug, about 99% of the applied amount remained in the donor solution after the experiment. SA, the most hy-

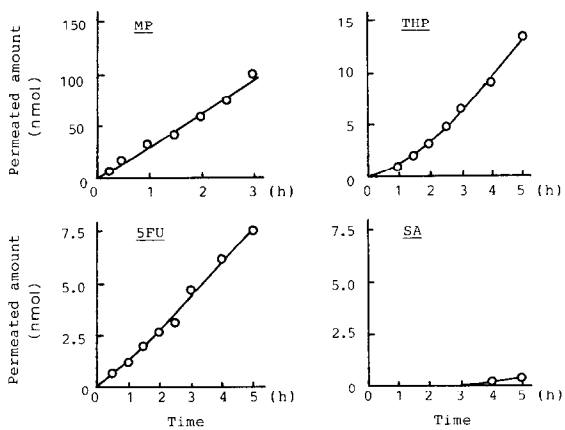

Fig. 4. Time course of drug permeation across the SC. Initial drug concentration on the donor side: 1 mM, MP; 10 mM, THP; 10 mM, 5FU; 20 mM, SA. Each point in all four panels represents the mean of at least three experiments.

drophilic drug which is completely ionized at pH 7.4, scarcely permeated through the SC under control conditions.

The K_p values of the drugs investigated to the SC under various conditions are listed in Table 1, together with the corresponding molecular weights and *n*-octanol/water partition coefficients at pH 7.4. Under control conditions, the K_p values of drugs were of the same order of magnitude as their partition coefficients.

Effects of Azone and SLS on the R_m of SC and its permeability

The effects of Azone and SLS on the R_m of the SC are depicted in Figs 5 and 6, respectively. Marked differences were observed between the effects of both adjuvants on R_m and on the permeability of the SC to drugs (Table 1). Pretreatment with Azone only slightly accelerated the decline in R_m value of the SC, regardless of its concentration. This effect was not due to polysorbate 20, an emulsifying agent contained in the solution with Azone. In contrast, SLS gave rise to a rapid fall in the value of R_m, its effect on this parameter being observed to depend on the concentration. At 20 mM SLS, R_m diminished to a value within the range of 0.3–0.5 Ωcm^2.

Pretreatment of the SC with 20 mM SLS resulted in a significant enhancement of the value for K_p for all four drugs. SLS was found to have the greatest influence in the case of hydrophilic drugs such as 5FU and SA. However, 5% Azone had the strongest effect on the K_p value of THP

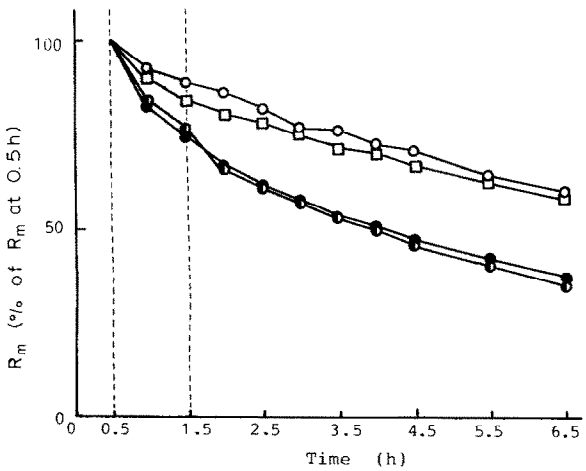

Fig. 5. Effect of Azone on R_m of the SC. Values are expressed as % of R_m measured before pretreatment (at 0.5 h). SC pretreated with standard Ringer solution (□) or Ringer solution containing only 0.1% polysorbate 20 (○), 1% Azone (◐) or 5% Azone (●). Pretreatment period (for 1 h) indicated by stippled area. Each point represents the mean of at least three experiments.

although its influence on 5FU and SA was minor as compared with that of SLS.

Fig. 7 represents the relationship between K_p of THP and the electrical conductance (G_m) of the SC under various conditions. G_m was calculated as the reciprocal of R_m and expressed in units of mS/cm^2. In Fig. 7, $\overline{G}_m$ represents the mean value of G_m at 3, 4 and 5 h after the end of pretreatment, since under all conditions, the flux rates of THP during this period remained constant and were used in the calculation of K_p.

TABLE 1

Effect of Azone (5%) and SLS (20 mM) on K_p of various drugs

Drug	Molecular weight	PC [a]	K_p ($\mu l/cm^2$ per h)		
			Control	5% Azone	20 mM SLS
MP	152.2	93.0	43.40 ± 5.2	111.10 ± 8.9	102.60 ± 5.0
THP	180.2	0.964	0.44 ± 0.67	3.26 ± 0.13	3.22 ± 0.15
5FU	130.0	0.116	0.22 ± 0.04	0.44 ± 0.04	2.50 ± 0.99
SA	173.2	0.007	n.d. [b]	0.03 ± 0.01	0.99 ± 0.10

[a] Apparent partition coefficient of drug was determined in an *n*-octanol/Ringer solution (pH 7.4) system at 37°C.

[b] Amount of SA permeated was too small to calculate the K_p under control conditions (Fig. 4). Each value of K_p is expressed as the mean ± S.E. of at least three experiments.

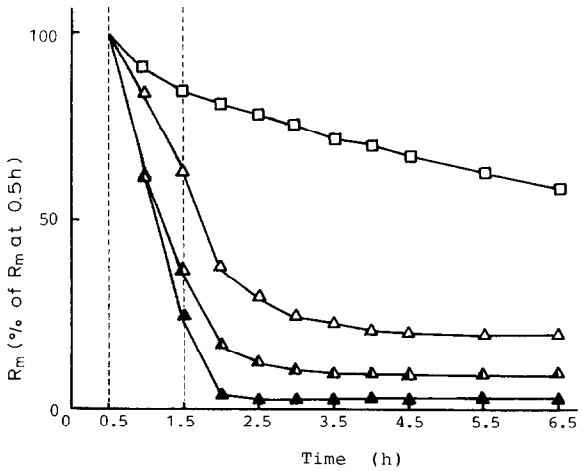

Fig. 6. Effect of SLS on R_m of the SC. Values are expressed as % of R_m measured before pretreatment (at 0.5 h). SC pretreated with standard Ringer solution (□) or Ringer solution containing 2 mM SLS (△), 5 mM SLS (▲) or 20 mM SLS (▲). Pretreatment period (for 1 h) indicated by stippled area. Each point represents the mean of at least three experiments.

Each point in Fig. 7 corresponds to the K_p and $\overline{G}_m$ of each SC. As shown above in Fig. 5, SLS caused a marked decrease in R_m and thus the increase in $\overline{G}_m$ shown in Fig. 7. A linear relationship was observed between $\overline{G}_m$ and K_p of THP with a high regression coefficient ($R = 0.99$), until $\overline{G}_m$ of the SC had attained a level of about 1.0 mS/cm^2. On raising the concentration of SLS to 20 mM, this point deviated from the regression line. On the other hand, Azone increased the permeability to THP to the same level as that in the case of 20 mM SLS without a significant change in $\overline{G}_m$. In this instance, no correlation between K_p of THP and $\overline{G}_m$ was observed. This would appear to indicate that Azone enhanced the permeability to THP in a manner different from that with SLS.

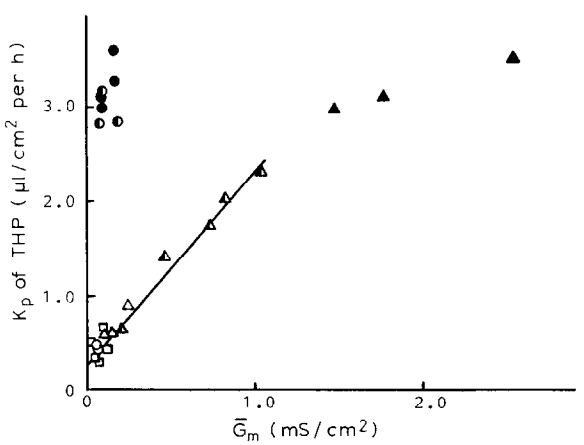

Fig. 7. Relationship between $\overline{G}_m$ and K_p of THP. Each point represents $\overline{G}_m$ and K_p of THP in each SC. Symbols defined as in Figs 5 and 6. Regression line was obtained from the results of control, 2 mM SLS and 5 mM SLS experiments.

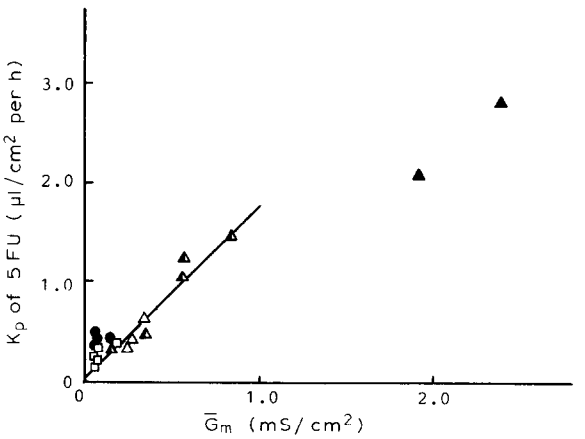

Fig. 8. Relationship between $\overline{G}_m$ and K_p of 5FU. Each point represents $\overline{G}_m$ and K_p of 5FU in each SC. Symbols as defined in Figs 5 and 6. Regression line was obtained from the results of control, 2 mM SLS and 5 mM SLS experiments.

Similar results were obtained for 5FU, which is more hydrophilic than THP, although Azone showed little effect on the K_p of 5FU (Fig. 8). On pretreatment with SLS, a linear relationship between $\overline{G}_m$ and K_p was also observed for 5FU ($R = 0.97$). The straight line in Fig. 8 has a similar slope to that in Fig. 7 (2.05 ± 0.09 for THP, 1.73 ± 0.14 for 5FU).

Discussion

We have demonstrated previously that the SC of hamster cheek pouch plays a major role as a barrier to the absorption of salicylic acid (Kurosaki et al., 1989). Isolated sheets of this SC could be used for in vitro examination, since no appendages (hair follicles or sweat ducts) are present in this region. Moreover, the structure of

the SC of hamster cheek pouch is similar to that of the skin, i.e., of the 'brick-and-mortar' type where keratin-rich cells are embedded in lipids to construct multiple layers (White and Gohari, 1981). Although some differences in thickness or permeability were found, the isolated SC of hamster cheek pouch was considered to be one of the most readily available model membranes for the SC of skin. With it, we can evaluate the primary interaction of the percutaneous absorption enhancer with the SC itself, without taking into account contributions of any appendages or deeper layers (epidermis or dermis).

In addition to the permeability to drugs of the membrane, we measured the electrical resistance (or the conductance) of the membrane. Since current flow is mediated by the movement of current-carrying ions (mainly Na^+ and Cl^-), R_m represents the resistive function of the membrane against ion flow and, thus, the parameter G_m is proportional to the ion permeability of the membrane. The R_m value of the SC was about 400–500-fold greater than that of the gastrointestinal epithelial membrane (Powell, 1981) and about 80% of that of intact cheek pouch. These findings suggest that the isolated SC of hamster cheek pouch maintains an adequate degree of function as a barrier to the permeation of substances, especially polar species. The relatively low K_p values of hydrophilic drugs (Table 1) support this suggestion. In other words, isolated SC of hamster cheek pouch behaves as a lipid barrier to drug permeation, precisely as the SC of the intact skin.

SLS, an anionic surfactant with an alkyl chain length of 12, is well known to enhance the percutaneous absorption of several diverse chemicals (Walters, 1988). As shown in Figs 7 and 8, the enhancement of permeability to THP or 5FU induced by SLS was closely correlated with the increase in G_m, suggesting that the permeability-enhancing effect of SLS was due to the reduced barrier function of SC against ion flow. It has been reported that SLS mainly interacts with the protein fraction within the epidermis and promotes its hydration (Breuer, 1979; Rhein et al., 1986). Dugard et al. (1973) demonstrated that SLS produced the largest increase in G_m of human epidermis among a homologous series (C_8–C_{16}) of surfactants. They suggested that its action on the skin was profoundly related to the ability to interact with and bind to the epidermal protein. Therefore, it was believed that the SLS-induced hydration and disorder in the protein matrix within the keratinocyte made the SC more permeable to ions, resulting in a decrease in R_m and enhancement of drug permeability.

Azone is an adjuvant which was designed and synthesized for the purpose of enhancing the percutaneous absorption of drugs. It has been shown to exert a distinct effect on the percutaneous absorption of various drugs (Stoughton, 1982; Stoughton and McClure, 1983). However, the mechanism of action of Azone has not yet been clarified sufficiently. In our study, Azone differed in action from SLS in the following aspects: (i) Azone mainly enhances permeability to lipophilic drugs; (ii) Azone results in only a slight decrease in R_m; and thus (iii) the permeability-enhancing effect of Azone is independent of the increase in G_m. Goodman et al. (1985) demonstrated that Azone mainly interacts with lipid components within the human SC, thereby disrupting the structure of the lipid layer. We have also reported that Azone increases the fluidity of lipids extracted from the SC of hamster cheek pouch (Kurosaki et al., 1989). Consequently, it is reasonable to consider that the primary action of Azone is localized in the lipid region within the SC. Elias et al. (1981, 1983) suggested that a major role is played by the extracellular lipids as a pathway for fairly lipophilic substances. In our experiments, Azone could also interact with the extracellular lipids of the SC, increasing its fluidity and enhancing its permeability to fairly lipophilic drugs. Since the step of partitioning into the lipid layer is necessary for progressing through this route, polar substances such as ions are scarcely able to traverse it. This suggestion can explain the observation that Azone exerts little effect on R_m and the permeability to 5FU and SA, which barely partition into the lipid layer due to their low lipophilicity.

Morimoto et al. (1986) demonstrated that Azone markedly (about 100-fold) enhanced the permeability of hairless rat skin to 5FU, although

its effect was detectable only after a considerable lag time of approx. 10 h had elapsed. Apart from the difference in origin of the membrane, this inconsistency in the observed effects of Azone could arise from a difference in duration of the period of Azone treatment. It was believed that Azone firstly partitions into the lipid layer thereby enhancing lipid fluidity, followed by exerting a secondary effect which probably induces structural changes in other regions within the SC, including that of some appendages. The enhanced permeability to 5FU detected after a prolonged lag time might correspond to the secondary action of Azone. An electrophysiological study using animal skin instead of isolated SC is necessary in order to elucidate this problem. Investigations of this nature are currently underway.

Various methods have been employed in the evaluation of drug permeability of skin under in vivo or in vitro conditions. Such techniques can also be utilized to detect the ability of adjuvants to enhance drug absorption. However, with respect to the manner in which an enhancer interacts with skin, a parameter which can provide information concerning the change in function or structure of the skin is necessary. Okamoto et al. (1988) derived a diffusion parameter and a partition parameter which represent the drug diffusivity in the skin and drug affinity to the skin, respectively, based on a single-layer diffusion model. They successfully characterized the process of enhancement of skin permeability to drugs induced by several adjuvants with the above parameters. Barry (1987) clarified the interaction of enhancer with a number of components of human SC in a differential scanning calorimetry study and proposed a possible mode of action for various enhancers. In the present investigation, we measured the electrical resistance of the SC at the same time as determining the degree of drug permeation. This makes it possible to evaluate the change in barrier function of the SC quickly and, further, to correlate the changes in permeability and resistance directly. Such information should be useful in the discussion of the mechanism of action of enhancers. This method is considered to be widely applicable to the characterization of interactions between many other adjuvants and the SC.

References

Barry, B.W., Mode of action of penetration enhancers in human skin. *J. Controlled Release*, 6 (1987) 85–97.

Barry, B.W., Optimizing percutaneous absorption. In Bronaugh, R.L. and Maibach, H.I. (Eds), *Percutaneous Absorption*, Dekker, New York, 1989, pp. 531–554.

Breuer, M.M., The interaction between surfactants and keratinous tissue. *J. Soc. Cosm. Chem.*, 30 (1979) 41–64.

Catz, P. and Friend, D.R., Alkyl esters as skin permeation enhancers for indomethacin. *Int. J. Pharm.*, 55 (1989) 17–23.

Dugard, P.H. and Scheuplein, R.J., Effects of ionic surfactants on the permeability of human epidermis: an electrometric study. *J. Invest. Dermatol.*, 60 (1973) 263–269.

Elias, P.E., Cooper, E.R., Korc, A. and Brown, B.E., Percutaneous transport in relation to stratum corneum structure and lipid composition. *J. Invest. Dermatol.*, 76 (1981) 297–301.

Elias, P.E., Epidermal lipids, barrier function, and desquamation. *J. Invest. Dermatol.*, 80 (1983) 44–49.

Evans, T., Griffith, S.G., Kohn, P.G., Mckeever, A., Siddons, B.M., Simpson, J., Stobbs, M., Syme, G.B. and Wilson, E.L., Preliminary study on electrical properties of hamster cheek pouch. *J. Dent. Res.*, 58 (1979) 1265 (Abstr.).

Goodman, M. and Barry, B.W., Differential scanning calorimetry (DSC) of human stratum corneum: effect of Azone. *J. Pharm. Pharmacol.*, 37 (1985) 80.

Kadir, R., Stempler, D., Liron, Z. and Cohen, S., Penetration of theophylline and adenosine into excised human skin from binary and ternary vehicles: effect of a nonionic surfactant. *J. Pharm. Sci.*, 78 (1989) 149–153.

Kimura, T., Kim, K.S. and Sezaki, H., Effect of taurine on drug absorption from the rat gastrointestinal tract. *J. Pharmacobio-Dyn.*, 4 (1981) 35–41.

Kurosaki, Y., Aya, N., Okada, Y., Nakayama, T. and Kimura, T., Studies on drug absorption from oral cavity: physicochemical factors affecting absorption from hamster cheek pouch. *J. Pharmacobio-Dyn.*, 9 (1986) 287–296.

Kurosaki, Y., Hisaichi, S., Hong, L.Z., Nakayama, T. and Kimura, T., Enhanced permeability of keratinized oral-mucosa to salicylic acid with 1-dodecylazacycloheptan-2-one (Azone). In vitro studies in hamster cheek pouch. *Int. J. Pharm.*, 49 (1989) 47–55.

Morimoto, Y., Sugibayashi, K., Hosoya, K. and Higuchi, W.I., Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin. *Int. J. Pharm.*, 32 (1986) 31–38.

Okamoto, H., Hashida, M. and Sezaki, H., Structure-activity relationship of 1-alkyl- or 1-alkenylazacycloalanone derivatives as percutaneous penetration enhancers. *J. Pharm. Sci.*, 77 (1988) 418–424.

Powell, D.W., Barrier function of epithelia. *Am. J. Physiol.*, 241 (1981) G275–G288.

Reid, C.O., Hardcasyle, J. and Smith, J., A comparison of some of the permeability characteristics of intact and tape-stripped hamster cheek pouches in vitro. *J. Dent. Res.*, 65 (1986) 673–676.

Rhein, L.D., Robbins, C.R., Fernee, K. and Cantore, R., Surfactant structure effects on swelling of isolated human stratum corneum. *J. Soc. Cosm. Chem.* 37 (1986) 125–139.

Stoughton, R.B., Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. *Arch. Dermatol.*, 118 (1982) 474–477.

Stoughton, R.B. and McClure, W.O., Azone: a new nontoxic enhancer of cutaneous penetration. *Drug Dev. Int. Pharm.*, 9 (1983) 725–744.

Tregar, R.T., *Physical Function of Skin*, Academic Press, London, 1966, Chap. 2.

Walters, K.A., Penetration enhancers and their use in transdermal therapeutic system. In Swarbrick, J. (Ed.), *Transdermal Drug Delivery*, Dekker, New York, 1988, pp. 197–246.

White, F.H. and Gohari, K., The ultrastructural morphology of hamster cheek pouch epithelium. *Arch. Oral Biol.*, 26 (1981) 563–576.

Yamashita, S., Saitoh, H., Nakanishi, K., Masada, M., Nadai, T. and Kimura, T., Effects of diclofenac sodium and disodium ethylenediaminetetraacetate on electrical parameters of the mucosal membrane and their relation to the permeability enhancing effects in the rat jejunum. *J. Pharm. Pharmacol.*, 39 (1986) 621–626.

Yamashita, S., Suda, Y., Masada, M., Nadai, T. and Sumi, M., 5-Fluorouracil derivatives with serum protein binding potencies. *Chem. Pharm. Bull.*, 37 (1989) 2861–2863.