Effect of Benzyl Alcohol on Rat Skin as a Solvent of Liquid Droplet Dispersion Ointment

Izumi SAITOH,^{*,a} Kaori IKEDA,^a and Yasushi TAKAGISHI^b

Developmental Research Laboratories, Shionogi & Co., Ltd.,^a 2-1-3, Kuise-Terajima, Amagasaki, Hyogo 660, Japan
and Developmental Research Laboratories, Shionogi & Co., Ltd.,^b 5-12-4, Sagisu, Fukushima-ku, Osaka 553, Japan.

Received July 11, 1994; accepted October 14, 1994

The action of benzyl alcohol (BA) as a major solvent of a liquid droplet dispersion ointment (LDDS), a preparation enabling excellent percutaneous absorption of drugs, was evaluated and compared with Azone and dimethyl sulfoxide (DMSO), which are known percutaneous absorption enhancers. Using a water sorption-desorption test, BA was found to increase hygroscopicity and decrease water-holding capacity to the same extent as Azone and DMSO. Differential scanning calorimetry (DSC) determination of the whole stratum corneum, and its lipids and proteins confirmed that BA and Azone act on the stratum corneum lipids while DMSO acts on its lipids and proteins. Furthermore, DSC and X-ray diffraction spectrum determinations of lipids in the stratum corneum suggest that the action of BA is moderate and reversible. The effect of BA may be one of the factors underlying the high percutaneous absorption found with LDDS.

Key words benzyl alcohol; skin; stratum corneum; lipid; enhancer; rat

We have reported that percutaneous absorption of a drug from a liquid droplet dispersion ointment (LDDS) was largely due to high release of the drug from this formulation.¹⁾ We also reported that the drug concentration in a continuous phase was closely related to syneresis of LDDS, and that the absorption of benzyl alcohol (BA) used in this formulation as a solvent improved drug permeability.²⁾ Azone and dimethyl sulfoxide (DMSO) accelerate percutaneous absorption of a drug by acting on lipids in the stratum corneum.³⁾ Similar reports for ethanol, when admixed with LDDS,⁴⁾ prompted us to evaluate the action of BA in comparison with Azone and DMSO.

MATERIALS AND METHODS

Materials and Reagents BA as a solvent for LDDS, DMSO (Wako Pure Chemical Industries, Tokyo, Japan; analytical grade) and Azone (Nelson Research, Irvine, CA, U.S.A.) were used as enhancers. For all other reagents, the analytical grade or HPLC grade was selected.

Water Sorption-Desorption Test Approximately 9 week-old, male Wistar rats (weight 260–300 g) were held in a fixed position and anesthetized with urethane. The abdominal fur was clipped and shaved with an electric hair clipper and shaver, then the abdominal skin was cleaned with warm water. Markings were applied to five sites on the abdominal region, and a water sorption-desorption test was performed, using a skin surface hydrometer (IBS Inc., Tokyo, Japan; Skikon-200) following the method of Tagami *et al.*⁵⁾ The data obtained from this test were assessed using Student's *t*-test. A *p* value less than 0.05 was considered to be statistically significant.

Separation of the Stratum Corneum and Extraction of Lipids Depilated abdominal skin specimens were obtained from rats treated as described above. The stratum corneum was separated by the method of Swartzendruber *et al.*⁶⁾ Lipids were extracted from the stratum corneum by the method of Abraham and Downing,⁷⁾ and its residues were used as protein components.

Differential Scanning Calorimetry (DSC) Determination of the Stratum Corneum, Lipids and Protein Fractions

Samples of 10 mg each of stratum corneum, lipids and protein fractions were placed in closed type DSC measurement cells (volume 50 μ l) to be hydrated up to approx. 50% by the method of Goodman and Barry,⁸⁾ and 20 μ l of each enhancer was added. The cell was left standing for 2 h and excess enhancer was absorbed by filter paper. The cell was then tightly closed to produce the samples. These samples were pre-cooled for 30 min at -5°C in a DSC instrument (Seiko I & E, Ltd., Tokyo, Japan; Type DSC 200) and then subjected to DSC analysis under temperature which increased at a rate of $3^{\circ}\text{C}/\text{min}$ from 0 to 170°C .

Separation of Lipid Components by Solid Extraction and TLC Analysis The obtained lipids were separated into fraction A of polar components and fraction B of the other components by the solid extraction method of Kaluzny *et al.*⁹⁾ Fraction A was subjected to two kinds of TLC analysis using a two-stage development method with the following solvents:

condition A: 1st developer benzene:hexane = 1:1,
2nd developer hexane:ethyl ether:acetic acid
= 70:30:1

condition B: 1st developer chloroform:ethanol:water = 90:10:1,
2nd developer petroleum ether:ethyl ether:acetic acid = 70:50:1

X-Ray Diffraction Spectrum Measurement of Lipids in the Stratum Corneum

Samples of the obtained lipids were loaded in the X-ray diffraction measurement liquid-use cells (Rigaku Co., Tokyo, Japan) and were hydrated up to approx. 50% by the method of Goodman and Barry.⁸⁾ The respective enhancers were added, and then the cells were allowed to stand for 2 h. Excess enhancer was absorbed by filter paper, and the X-ray diffraction spectra were measured at a speed of $4^{\circ}/\text{min}$ over the range of $2\theta = 4^{\circ}$ – 50° , using an X-ray diffractometer (Rigaku Co., Tokyo, Japan; Type Rint-1100).

* To whom correspondence should be addressed.

RESULTS

Changes of the Stratum Corneum Function by Enhancers Since Azone and DMSO are known to act on the stratum corneum of the skin, we compared the effects of each enhancer on the hygroscopicity of the stratum corneum by performing a water sorption-desorption test.

Fig. 1. Hygroscopicity and Water-Holding Capacity of Rat Skin with DMSO, Azone and BA

■, initial (1st trial); ●, 0.5 h (2nd); ▲, 2.5 h (3rd); ◆, 4.5 h (4th); pre, the value before treatment. a: no treatment, b: DMSO treatment, c: Azone treatment, d: 3% Azone emulsion treatment, e: BA treatment. Each value indicates the mean $\pm$ S.D. of 5 measurements.

Figure 1a shows the results of this test for a control (no enhancer). No differences were found between hygroscopicity and the water-holding capacity of the stratum corneum up to 4.5 h. In contrast, as shown in Fig. 1b, treatment with DMSO led to a considerable increase in the water content of the stratum corneum, and edema was observed. Consequently, hygroscopicity and the water-holding capacity could not be evaluated at this time. The considerable increase of water content in the stratum corneum disappeared 2.5 h after treatment, thereafter the hygroscopicity and water holding capacity were significantly lower. As shown in Fig. 1c, Azone treatment resulted in a considerable increase of the stratum corneum water content immediately after the treatment, and even after 4.5 h, hygroscopicity levels remained at 80% of the value registered immediately after treatment. However, the water-holding capacity dropped to levels almost equal to pre-treatment values at 2.5 h after treatment. Similarly, treatment with 3% Azone emulsion led to an increase of 60% hygroscopicity and 30% water-holding capacity as compared with the Azone treatment (Fig. 1d). The pattern of decrease was similar to that observed with Azone. As shown in Fig. 1e, BA treatment induced changes similar to those observed with 3% Azone emulsion, and both water-holding capacity and hygroscopicity increased just after BA treatment. However, BA treatment led to an extended period of hygroscopicity and to a significant decrease of water-holding capacity 2.5 h after treatment.

Elucidation of Action of Enhancers on Cutaneous Stratum Corneum by DSC Analysis The above described findings reveal that BA, Azone and DMSO act on the cutaneous stratum corneum. Goodman has shown, by DSC determination, that Azone and DMSO alter the DSC profiles of lipids in the human cutaneous stratum corneum.⁸⁾ As it is presumed that BA also has a similar action on lipids in the stratum corneum, we evaluated the action of these substances by DSC analysis. Figure 2 shows the DSC profiles of untreated stratum corneum, lipids and proteins. Analysis of the data indicates that a series of peaks from 20 to 80 °C are probably induced by lipids, while the peaks around 140 °C are from proteins. The reproduced peaks obtained upon the 2nd measuring (re-heating) suggest that the lipid-induced peaks are accompanied by either fusion or phase transition, while the protein-induced peaks are due to denaturation.

The actions of DMSO on the stratum corneum, lipids and proteins having such DSC profiles are shown in Fig. 3-1. In the stratum corneum, DMSO causes the disappearance of all the peaks induced by lipids and proteins

Fig. 2. DSC Profile of the Stratum Corneum, and of Its Lipids and Proteins

a: the stratum corneum, b: lipids, c: proteins, upper: 1st measurement, lower: 2nd measurement (re-heating).

Fig. 3. DSC Profile of the Stratum Corneum, and Its Lipids and Proteins Treated by DMSO, Azone and BA

1: DMSO treatment, 2: Azone treatment, 3: BA treatment. a: stratum corneum, b: lipids, c: proteins, upper: no treatment, lower: treatment.

Fig. 4. DSC Profiles of Lipids and Separated Fractions

a: lipids, b: fraction A separated from lipids, c: fraction B separated from lipids, d: mixture of fraction A and B.

without treatment and induces masked peaks at 30, 70 and 120 °C. This tendency is similar to the result of Goodman. In the DSC profiles of lipids (Fig. 3-1), the peaks seen at around 60 °C without treatment were observed to shift to a lower temperature range. Moreover, a significant peak was observed for protein (Fig. 3-1). In contrast, as shown in Fig. 3-2, Azone did not demonstrate a strong effect on the stratum corneum itself, and showed hardly any effect on protein components. Azone did, however, cause the lipid component peaks observed at around 60 °C without treatment to either disappear or shift to a lower temperature range. As shown in Fig. 3-3, BA also demonstrated similar changes in the peaks of the stratum corneum and lipids as in the case of Azone, but did not induce any changes in protein components.

Elucidation of BA Action on Lipids in the Stratum Corneum DSC profiles of lipids in the stratum corneum

have revealed two characteristic peaks at around 40 and 60 °C, and BA was confirmed to act on peaks in the higher temperature range. We thus separated each component fraction corresponding to each of the two peaks using the solid extraction method.⁹⁾ Figure 4 shows the DSC profiles of the two fractions separated from lipids in the stratum corneum. Even after re-mixing, the two peaks seen in the DSC profiles obtained by the solid-liquid extraction method were almost completely separated and were confirmed to return to almost the original state before separation. Figure 5 shows the DSC profiles at the time when BA was applied to the separated fractions. BA shifted the peak of fraction A to a lower temperature, but hardly had any effect on fraction B. The results of TLC development of fraction A are shown in Fig. 6. Fraction A was found to contain ceramide, cholesterol, mono- and di-glyceride which are all polar lipids.

Transient Effect of BA on Lipids in the Stratum Corneum

BA was confirmed to act on polar lipids in the stratum corneum. If this effect is due to a permanent change in the structure of lipids, it would be reasonable to expect the function of the stratum corneum to have been disturbed. We therefore evaluated the transient effect of BA on lipids. Figure 7 shows a comparison between two DSC profiles; one at the time when BA was applied to lipids in the stratum corneum, and the other when BA was first applied to lipids in the stratum corneum and then removed by the solid extraction method. The lipids in the stratum corneum treated with BA, once removed from the system, showed identical DSC profiles with those before BA treatment. Lipids in the stratum corneum, even after extraction, retain this lamella structure.¹⁰⁾ Figure 8 shows a comparison of X-ray diffraction spectra between lipids treated with BA and lipids treated with DMSO. Two peaks

Fig. 5. DSC Profile of the Fraction A and B Effect Treated by BA
a: fraction A, b: fraction B, upper: no treatment, lower: BA treatment.

Fig. 6. TLC Pattern of Fraction A

St, standard sample; A, fraction A. 1: condition A, 2: condition B. Abbreviations; SQ, squarene; WE, wax ester; CE, cholesteric ester; FFA, fatty acid; TG, triglyceride; DG, diglyceride; MG, monoglyceride; C, cholesterol; PL, phospholipids; CER, ceramide; GC, glycolipids; SM, sphingomyerin.

Fig. 7. Transient Action of BA on the Stratum Corneum Lipids
a, before BA treatment; b, after BA treatment; c, after removal of BA.

that are presumed to be caused by the lamella structure of lipids were hardly affected by BA treatment, but their peak intensities were reduced by DMSO treatment.

Fig. 8. X-Ray Diffraction Spectrum of Lipids Treated with BA or DMSO

a, no treatment; b, treated with BA; c, treated with DMSO.

DISCUSSION

Evaluation of Changes in the Function of the Cutaneous Stratum Corneum by Water Sorption-Desorption Test

Treatment of the skin with a chemical substance can reduce the hygroscopicity of the stratum corneum.¹¹⁾ Tagami *et al.*⁵⁾ and Imokawa¹²⁾ have already reported that these changes can be evaluated by determining the water content within the stratum corneum. Thus, the changes in the function of the stratum corneum have been evaluated from the changes in its hygroscopicity and water-holding capacity.¹³⁾ According to Takenouchi *et al.*,¹⁴⁾ water in the stratum corneum can be classified into 1st bound water, 2nd bound water and free water. In the water sorption-desorption test of the stratum corneum, hygroscopicity is associated with free water, while the water-holding capacity is associated with 1st bound water. The large, transient increase of water in the stratum corneum treated with DMSO as shown in Fig. 1 may be due to an abnormal increase of free water and 1st bound water in the stratum corneum, which suggests that there are large changes caused by DMSO treatment. Azone and BA treatments, as shown in Fig. 1, contributed to hygroscopicity, and the influence of these substances on the stratum corneum was smaller than that of DMSO. The higher potency of Azone toward hygroscopicity than that of BA suggests that the effect of Azone on the stratum corneum is stronger than

that of BA. However, because of the larger BA-induced decline in water-holding capacity than that caused by Azone, BA is considered to be stronger than Azone in its action on the lipids themselves.

Evaluation of Enhancers on the Stratum Corneum by DSC The evaluation of the physicochemical properties of the cutaneous stratum corneum by DSC was initiated by Wilkes *et al.*,¹⁵⁾ and has since been adopted by several investigators. Studies have confirmed that in mammals such as humans and rats, there exists an endothermic peak which is regarded as a phase transition of lipids at around 40 and 60 °C.¹⁶⁾ The increased intensity of peaks in DSC profiles of the stratum corneum suggests that extracted lipids maintain a structure nearly identical to the lipid structure of the stratum corneum. In this regard, Abrahams and Downing⁷⁾ reported the ability to retain the lamella structure of lipids. Assessing this endothermic peak at the time of DSC measurement of the stratum corneum, Goodman and Barry⁸⁾ reported that Azone and DMSO alter the peak intensity, and Khan and Kellaway¹⁷⁾ corroborated these results for DMSO. However, the DSC profiles shown in Fig. 3-1 clearly suggest that DMSO not only acts on lipids, but also on proteins. In contrast, as shown in Figs. 3-2 and 3-3, Azone and BA, unlike DMSO, do not act on proteins, but on lipids only. Moreover, both Azone and BA have been found to display a tendency to decrease peak intensity at higher temperature ranges and increase peak intensity at lower temperature ranges. This trend is found to be more marked with BA, suggesting that BA may act on lipids more strongly than Azone. This finding coincides with the results obtained from changes in the water-holding capacity.

Elucidation of Lipid Components Affected by BA and Its Transient Action From Fig. 5, it is clear that the two BA-induced endothermic peaks in the DSC profile of lipids in the stratum corneum are influenced by the shifting of a peak at the high temperature range. Therefore, the increased intensity of the peak in the lower temperature range in Fig. 4 was suggested to be caused by its shifting. The X-ray diffraction spectrum shown in Fig. 8 indicates that the lipid structure is maintained. Therefore, these peaks may be caused by phase transition, indicating that BA acts on lipids having a high phase transition point by reducing their transition temperature. Moreover, Fig. 7 suggests that BA acts on polar lipid components such

as ceramide, mono- and di-glyceride, and cholesterol. Imokawa *et al.*¹⁸⁾ have already shown that these lipids are from a lamellar structure in the gaps between cells of the stratum corneum. This finding appears to indicate that BA may act on lipids in the gaps between cells in the stratum corneum. Also, the shifted peak of fraction A in the DSC profile by the action of BA suggests that such action of BA on the lipids only loosens the lamellar structure, and does not destroy the structure, this is supported by the X-ray diffraction spectrum in Fig. 8. The reversibility of BA action on lipids, as illustrated in Fig. 8, suggests that the action of BA is transient and this can also be observed by the tendency of hygroscopicity to return.

REFERENCES

- 1) I. Saitoh, K. Ikeda, Y. Doi, S. Egawa, *Yakuzaigaku*, **50**, 286 (1990).
- 2) I. Saitoh, M. Takehara, Y. Takagishi, *Yakuzaigaku*, **54**, 35 (1994).
- 3) R. B. Stoughton, *Arch. Dermatol.*, **118**, 474 (1982); S. K. Chandrasekaran, P. S. Campbell, A. S. Michaels, *AIChE J.*, **23**, 810 (1977).
- 4) A. H. Ghanem, H. Mahmoud, W. I. Higuchi, U. D. Rohr, S. Borsadia, P. Liu, J. L. Fox, W. R. Good, *J. Controlled Release*, **6**, 75 (1987).
- 5) H. Tagami, Y. Kanamaru, K. Inoue, S. Suehisa, F. Inoue, K. Iwatsuki, K. Yoshikuni, M. Yamada, *J. Invest. Dermatol.*, **78**, 425 (1982).
- 6) D. C. Swartzendruber, D. J. Kitko, P. W. Wertz, K. C. Madison, D. T. Downing, *Arch. Dermatol. Res.*, **280**, 424 (1988).
- 7) W. Abraham, D. T. Downing, *Pharm. Res.*, **9**, 1415 (1992).
- 8) M. Goodman, B. W. Barry, *Anal. Proc.*, **23**, 397 (1986).
- 9) M. A. Kaluzny, L. A. Duncan, M. V. Merritt, D. E. Epps, *J. Lipid Res.*, **26**, 135 (1985).
- 10) A. G. Matolsky, *J. Invest. Dermatol.*, **67**, 20 (1976).
- 11) H. Tagami, *Nippon Koshohin Kagakkaishi*, **12**, 262 (1988).
- 12) G. Imokawa, *Nippon Koshohin Kagakkaishi*, **8**, 92 (1984).
- 13) J. L. Leveque (ed.), "Cutaneous Investigation in Health and Disease," Marcel Dekker Inc., New York, 1988, p. 79.
- 14) M. Takenouchi, N. Suzuki, Y. Okaya, M. Oyama, A. Kaneko, H. Tagami, *Nippon Koshohin Kagakkaishi*, **9**, 182 (1985).
- 15) G. L. Wilkes, A. L. Nguyen, R. Wildnauer, *Biochim. Biophys. Acta*, **304**, 267 (1973).
- 16) B. F. van Duzze, *J. Invest. Dermatol.*, **79**, 1 (1982); G. M. Golden, D. B. Guzek, R. R. Harris, J. E. McKie, R. O. Potts, *ibid.*, **86**, 255 (1986).
- 17) Z. U. Khan, I. W. Kellaway, *Int. J. Pharmaceut.*, **55**, 129 (1989).
- 18) G. Imokawa, S. Akasaki, A. Kawamata, S. Yano, N. Takaishi, *J. Soc. Cosmet. Chem.*, **40**, 273 (1989).