J. Pharm. Pharmacol. 1998, 50: 55–61
Received July 8, 1997
Accepted September 11, 1997

Effect of Ointment Bases on Topical and Transdermal Delivery of Salicylic Acid in Rats: Evaluation by Skin Microdialysis

TERUO MURAKAMI, MAKIKO YOSHIOKA, ICHIRO OKAMOTO, RYOKO YUMOTO, YUTAKA HIGASHI, KAYO OKAHARA* AND NOBORU YATA†

*Department of Biopharmaceutics, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734 and *Department of Dermatology, Tsuchiya General Hospital, 3-30 Nakashima-cho, Naka-ku, Hiroshima 730, Japan*

Abstract

Microdialysis has been used to determine the concentration of salicylic acid in skin tissue and plasma periodically for 4 h to evaluate the effect of ointment bases on topical and transdermal delivery of salicylic acid. The ointment bases examined were solbase (water-soluble), poloid and white petrolatum (oleaginous), hydrophilic poloid (water in oil (w/o) type emulsion lacking water) and absorptive ointment (w/o-type emulsion containing water). The ointments (0·1 g) containing 25 μmol salicylic acid were applied for 2 h to the surface of rat skin (1 cm^2) with (intact) or without the stratum corneum.

For intact skin, the extent of topical delivery from different ointments, evaluated by the area under the concentration–time curve (AUC) of salicylic acid in the skin tissue (AUC_{skin}), increased in the order solbase ≪ white petrolatum, poloid, hydrophilic poloid ≪ absorptive ointment. The ratio of AUC_{skin} (topical delivery) to the AUC of salicylic acid in plasma (AUC_{plasma}, transdermal delivery) varied remarkably among the different bases, the greatest ratio being observed for absorptive ointment. When the ointments were applied to skin surface without stratum corneum, AUC_{skin} for solbase was much higher (about 45 times that for intact skin), whereas only a small (two-fold) increase was observed for poloid and hydrophilic poloid and the increase was negligible for white petrolatum and absorptive ointment. For skin without the stratum corneum, the ratio AUC_{skin}/AUC_{plasma} for the different ointments was comparable, although the magnitudes of AUC_{skin} and AUC_{plasma} still varied substantially. The variance of AUC values arises as a result of the different rates of release of salicylic acid from the bases.

These results indicate that: the topical and transdermal delivery of salicylic acid in intact skin varies substantially among different ointment bases, and the greatest topical delivery is observed for absorptive ointment; use of absorptive ointment increases the retention of salicylic acid in the stratum corneum; and the stratum corneum functions strongly as a penetration barrier for solbase, moderately for poloid and hydrophilic poloid, and less for absorptive ointment and white petrolatum.

In evaluating the transdermal delivery of a drug in-vivo, the concentration profiles of a drug in blood (plasma), urine or both, or the profile of the amount of drug remaining on the skin surface, have been employed as standard methodology (Guy et al 1987). In these methods the use of labelled compounds is sometimes required if low plasma levels of the compounds are to be detected or if a long experimental period is necessary to recover the drug and its metabolite(s) in urine. Recently, however, in-vivo microdialysis has been accepted as a new technique for determining the unbound concentration of substances in the restricted extracellular space of tissues or biological fluids, because of its many advantages over other methods (Benveniste 1989; Benveniste & Huttemeier 1990; Elmquist & Sawchuk 1997). In the field of skin study, this technique has been used to monitor the time-course of chemical events in restricted skin

†Present address: Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-01, Japan.

Correspondence: T. Murakami, Department of Biopharmaceutics, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan.

tissues, e.g. the temporal release of histamine in cutaneous allergic reactions (Petersen et al 1992, 1994; Okahara et al 1995) and the change of serological parameters such as glucose level (Meyerhoff et al 1994; Sternberg et al 1994). Also, because of the high concentration of a drug in the restricted skin tissues, the topical or transdermal delivery of a drug has been readily evaluated by skin microdialysis without employing labelled compounds (Ault et al 1994; Matsuyama et al 1994a, b; Hegemann et al 1995).

We have previously evaluated the barrier function of the stratum corneum by determining the in-vitro permeability of intact skin, skin without the stratum corneum (tape-stripped skin) and lipid-extracted skin (Harada et al 1992, 1993). It was found that the barrier function of the stratum corneum to compounds with different lipophilicities varied substantially.

In this study the effect of different types of ointment base on topical and transdermal delivery of salicylic acid in relation to the role of the stratum corneum has been evaluated by determining the concentration of salicylic acid in skin tissues and plasma. The concentration of salicylic acid in the skin tissues was determined by skin microdialysis.

Materials and Methods

Materials

Salicylic acid was purchased from Wako Pure Chemicals (Osaka, Japan). Ointment bases used were of Japanese Pharmacopoeia XII grade. They were: solbase (water-soluble base; polyethylene glycol 4000 50%, polyethylene glycol 400 50%); poloid (oleaginous base; liquid paraffin 95%, polyethylene glycol 5%); white petrolatum (oleaginous base; white petrolatum 100%); hydrophilic poloid (water in oil (w/o) type emulsion lacking water; liquid paraffin 90·25%, polyethylene glycol 4·75%, glycerine fatty acid ester 5%); and absorptive ointment (w/o type emulsion containing water; white petrolatum 40%, lauromacrogol 0·5%, white beeswax 5%, sorbitan sesquioleate 5%, cetanol 10%, *p*-hydroxybenzoic acid ester 0·2%, purified water 39·3%). Other chemicals were of reagent grade and were used without further purification.

Animal studies

The dorsal skin hair of male Wistar rats, 230–270 g, was removed with clippers 1 day before the absorption study. The rat was anaesthetized with pentobarbitone (40 mg kg^{-1}; intra-peritoneal injection), fixed in a prone position on a surface kept at 37°C, and a femoral artery or a femoral vein, or both, was cannulated with polyethylene tubing (PE 50, Clay Adams, USA). Skin microdialysis was performed as described previously (Okahara et al 1995) by use of a microdialysis device obtained from Carnegie Medicine (Stockholm, Sweden). The length of the semi-permeable membrane of the microdialysis probe (CMA 10) was 4·0 mm with a molecular cut-off at 20 kDa. The microdialysis probe was implanted intra-cutaneously via a guide cannula (length 12·3 mm; outer diameter 1·05 mm). Tyrode solution (NaCl 0·8%, dextrose 0·1%, $NaHCO_3$ 0·1%, $CaCl_2$ 0·02%, KCl 0·02%, $MgCl_2$ 0·01%, $NaH_2PO_4.2H_2O$ 0·0066%) was perfused at a flow-rate of 3 L min^{-1} in single perfusion manner by means of a micro-infusion pump (CMA 100).

Recovery ratio of salicylic acid in the dialysate

The concentration of salicylic acid in the restricted skin tissues, the recovery ratio of salicylic acid in the dialysate, and the ratio of the concentration of salicylic acid in the dialysate to that in the skin tissues were determined as described previously (Nakazono et al 1992). Briefly, after implantation of the microdialysis probe in the dorsal skin of the anaesthetized rat, a solution of salicylic acid in pH 9 buffered saline was administered intravenously at a dose of 100 or 200 μmol kg^{-1} via a cannula inserted at a femoral vein, and dialysate and arterial blood were collected at designated times. The rat was killed by decapitation 90 min after the administration of salicylic acid and the skin tissues where the microdialysis probe was implanted were excised. The concentrations of salicylic acid in dialysate, plasma and skin tissues were determined as described below.

Topical application of salicylic acid ointments

Salicylic acid ointment was prepared by dispersing salicylic acid powder into ointment base at a concentration of 250 μmol g^{-1} by means of a mortar and pestle. After 30 min pre-perfusion of perfusate into an implanted microdialysis probe, salicylic acid ointment (0·1 g) was applied topically to the dorsal skin surface at a dose of 25 μmol salicylic acid cm^{-2} at the point where the microdialysis probe was cutaneously implanted. The skin surface was covered with polyethylene film during the experiment (occlusive dressing technique). The ointment applied was carefully wiped off 2 h after application. Dialysate and arterial blood were collected every 10 min for 4 h after application of ointment. A similar experiment was also performed on the skin surface without stratum corneum, the stratum corneum being removed by repeated stripping (20 times) with adhesive tape (Osamura et al 1984). Complete removal of the stratum corneum

was confirmed by optical microscopic observation of the skin section after haematoxylin-eosin staining.

Analysis

Analysis of salicylic acid was performed by fluorescence spectrophotometry at 298 nm for excitation and 407 nm for emission (F-3000 fluorimeter; Hitachi Ltd, Japan).

Dialysate (20 μL) was mixed with acetonitrile (0·4 mL) and NaOH (0·1 M; 1 mL) and the mixture analysed for salicylic acid. Plasma (50 μL) obtained by centrifugation of blood sample was mixed with distilled water (50 μL) and acetonitrile (0·6 mL) and the mixture was centrifuged at 3000 rev min^{-1} for 10 min. The supernatant (0·5 mL) was mixed with NaOH (0·1 M; 1 mL) and the mixture analysed for salicylic acid. To extract salicylic acid from skin tissues the excised tissue (0·1 g) was incubated in methanol–chloroform (2:1 v/v; 5 mL) for 2 h at 39°C in the same manner as for extraction of lipid from the skin (Harada et al 1992), then left to stand for one night at ambient temperature for further extraction. This procedure was repeated twice. The organic solvents were combined and evaporated under reduced pressure. Chloroform (4 mL) and HCl (0·1 M; 2 mL) were added to the residues and after shaking for 10 min the organic layer was separated by centrifugation. Salicylic acid in the organic layer (3 mL) was extracted with NaOH (0·1 M; 2 mL) and the aqueous layer (1 mL) was mixed with acetonitrile (0·5 mL) and analysed for salicylic acid.

Results

Recovery ratio of salicylic acid by skin microdialysis in-vivo

Salicylic acid disappeared slowly and mono-exponentially from plasma after intravenous administration at a dose of 100 or 200 μmol kg^{-1}. The concentration–time profile of salicylic acid in the skin dialysate during 30–90 min skin microdialysis after administration of salicylic acid was parallel with the plasma profile.

The recovery ratio of salicylic acid by microdialysis, estimated 90 min after administration from the ratio of the concentrations of salicylic acid in dialysate and excised skin tissue, is summarized in Table 1. There was no significant difference between recovery ratios measured for the two different doses of salicylic acid. The mean recovery ratio of salicylic acid by skin microdialysis was 4·84%.

Topical application of salicylic acid ointments

Three examples of the time-course of salicylic acid concentration in skin tissue and in plasma after topical application of different salicylic acid ointments on intact skin or skin without the stratum corneum are shown in Figures 1–3. The concentration of salicylic acid in the skin tissue was calculated by dividing the concentration of salicylic acid in the dialysate by 0·0484 (recovery ratio). After application of the ointments to intact skin the concentration of salicylic acid in skin tissue and plasma differed substantially depending on which ointment was applied. Concentrations were greatest for absorptive ointment (Figure 3). Some pharmacokinetic parameters obtained after application of salicylic acid to intact skin are summarized in Table 2. The extent of topical delivery of salicylic acid from different ointments, evaluated from the area under the concentration–time curve of salicylic acid in the skin tissue (AUC_{skin}), increased in the order solbase < white petrolatum, hydrophilic poloid, poloid ≪ absorptive ointment. On the other hand, the extent of transdermal delivery, evaluated from AUC_{plasma}, was in the order solbase, white petrolatum < poloid, hydrophilic poloid ≪ absorptive ointment. Accordingly, the ratio of AUC_{skin} to AUC_{plasma} varied for the different ointments. The ratio was greatest for the absorptive ointment.

Removal of the stratum corneum resulted in a substantial increase in topical and transdermal delivery from solbase (Figure 1), whereas the increase was only moderate for poloid (Figure 2) and hydrophilic poloid and less for white petrolatum and absorptive ointment (Figure 3). Some

Table 1. Concentration of salicylic acid in plasma, dialysate and skin 90 min after intravenous administration to rats.

Dose (μmol kg^{-1})	Plasma (μM)	Dialysate (μM)	Skin (μM)	Recovery (%)*
100	403 ± 34	7·6 ± 1·1	164 ± 14	4·17 ± 0·78
200	619 ± 19	15·8 ± 2·7	318 ± 14	4·93 ± 0·75

*Percent recovery by microdialysis was calculated by dividing the concentration of salicylic acid in the dialysate by that in the skin. Each value is the mean ± s.e.m. of results from three or four trials.

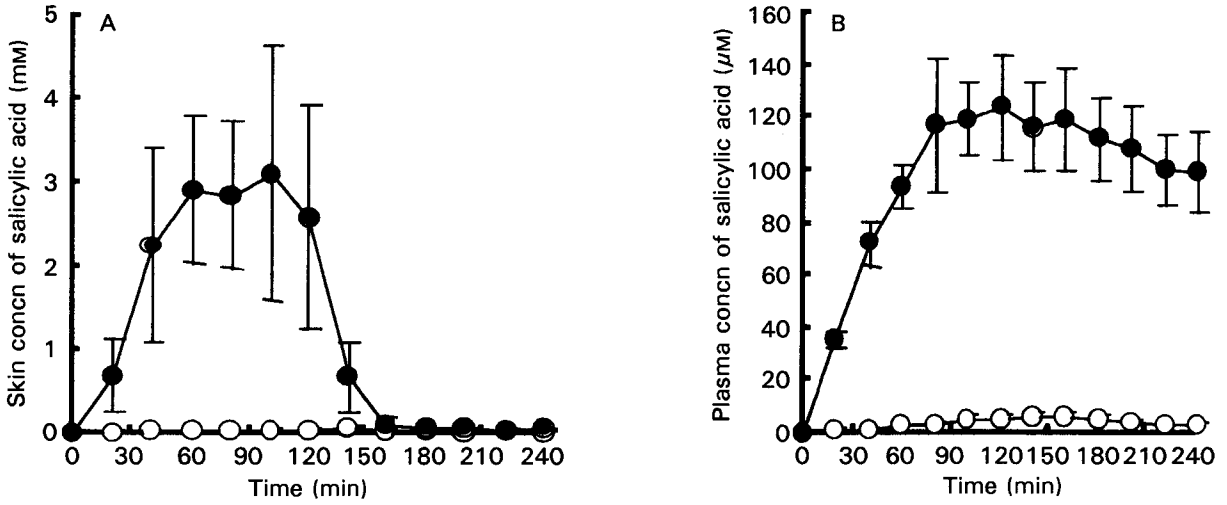

Figure 1. A. Concentration of salicylic acid in skin tissue and B. plasma after topical application of solbase containing salicylic acid to rat skin with (○) or without (●) the stratum corneum. The dose of salicylic acid was 25 μmol per rat. The ointment was removed 2 h after application. Each value is the mean ± s.e.m. of results from three or four trials.

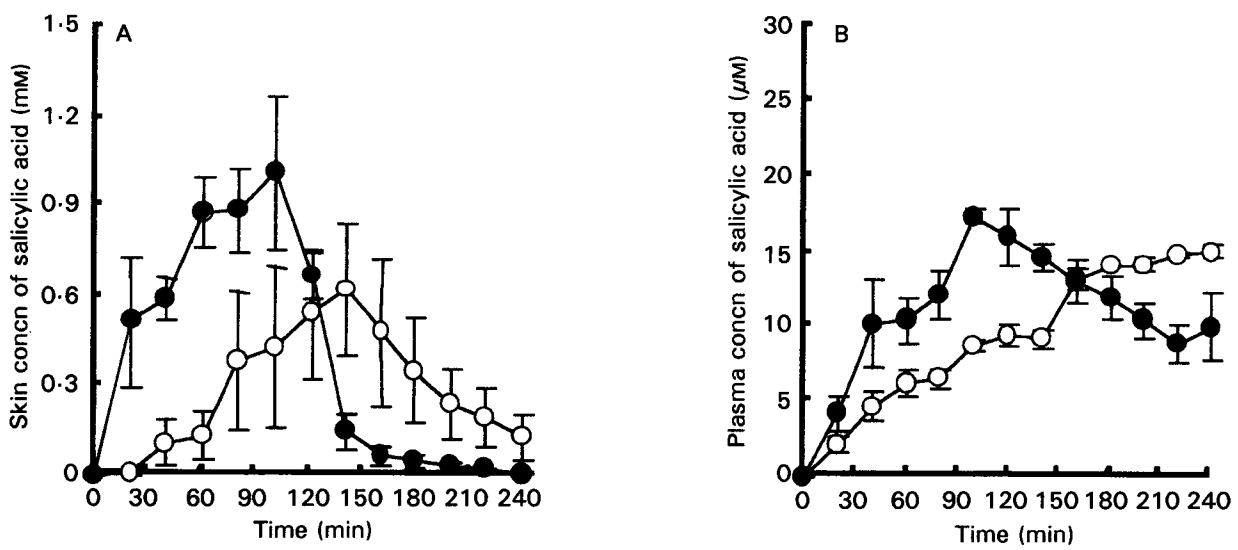

Figure 2. A. Concentration of salicylic acid in skin tissue and B. plasma after topical application of poloid containing salicylic acid to rat skin with (○) or without (●) the stratum corneum. The dose of salicylic acid was 25 μmol per rat. The ointment was removed 2 h after application. Each value is the mean ± s.e.m. of results from three or four trials.

pharmacokinetic parameters obtained after application of salicylic acid to skin without the stratum corneum are summarized in Table 3. Even for the skin without the stratum corneum, the magnitudes of AUC_{skin} and AUC_{plasma} varied substantially for the different ointments, although now the ratios of AUC_{skin}/AUC_{plasma} were similar for the different ointments, indicating that only the stratum corneum acts as a barrier. For comparison, skin tissue and plasma AUC_{skin}/AUC_{plasma} ratios for skin with and without the stratum corneum are summarized in Table 4. These ratios indicate that the stratum corneum functioned strongly as a penetration barrier to salicylic acid in solbase, moderately for poloid and hydrophilic poloid, and less so for absorptive ointment and white petrolatum.

Discussion

Topical and transdermal delivery of drugs by liposome carriers has recently become a topic of great interest; compared with other preparations liposomal delivery is reported to increase substantially the retention of drugs in the epidermis, including the stratum corneum (Foldvari 1994; Schmid & Korting 1994; Touitou et al 1994). For such delivery the determination of the concentration of a drug in skin tissue and plasma is essential

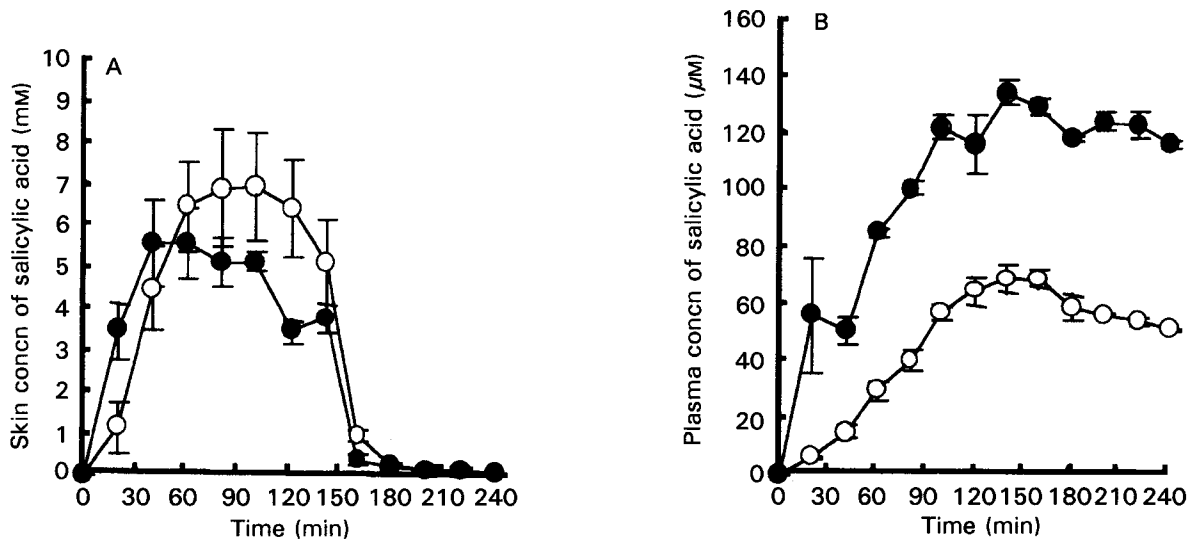

Figure 3. A. Concentration of salicylic acid in skin tissue and B. plasma after topical application of absorptive ointment containing salicylic acid to rat skin with (○) or without (●) the stratum corneum. The dose of salicylic acid was 25 μmol per rat. The ointment was removed 2 h after application. Each value is the mean ± s.e.m. of results from three or four trials.

Table 2. Topical and transdermal delivery of salicylic acid from different ointments through intact rat-skin.

	Solbase	Poloid	White petrolatum	Hydrophilic poloid	Absorptive ointment
Maximum concentration in skin (μM)	68 ± 29	622 ± 222	284 ± 40	586 ± 85	6972 ± 1275
Time of maximum concentration in skin (min)	140	140	160	40	100
Area under the skin concentration–time curve between 0 and 4 h (mM min)	6·8 ± 2·4	71·4 ± 36·0	38·6 ± 0·1	55·2 ± 5·5	793·4 ± 161·8
Maximum concentration in plasma (μM)	6·3 ± 1·6	15·0 ± 0·5	6·0 ± 3·8	27·5 ± 2·6	69·5 ± 5·1
Time of maximum concentration in plasma (min)	140	240	180	10	140
Area under the plasma concentration–time curve between 0 and 4 h (mM min)	0·9 ± 0·3	2·2 ± 0·1	0·9 ± 0·6	4·4 ± 0·8	10·9 ± 0·6
Ratio of the area under the concentration–time curve for skin to that for plasma	7·6	32·5	42·9	12·5	72·8

The dose of salicylic acid was 25 μmol. The concentration of salicylic acid in the skin was determined by microdialysis and from its percent recovery (4·84%). Each value is the mean ± s.e.m. of results from three or four trials.

Table 3. Topical and transdermal delivery of salicylic acid from different ointments through rat skin without stratum corneum.

	Solbase	Poloid	White petrolatum	Hydrophilic poloid	Absorptive ointment
Maximum concentration in skin (μM)	3142 ± 1526	1015 ± 255	270 ± 13	1200 ± 176	5664 ± 838
Time of maximum concentration in skin (min)	100	100	160	120	60
Area under the skin concentration–time curve between 0 and 4 h (mM min)	310 ± 20	98 ± 15	44 ± 1	140 ± 22	662 ± 111
Maximum concentration in plasma (μM)	125 ± 20	17 ± 0	10 ± 0	28 ± 2	135 ± 4
Time of maximum concentration in plasma (min)	120	100	180	100	140
Area under the plasma concentration–time curve between 0 and 4 h (mM min)	23·6 ± 3·2	2·7 ± 0·3	1·5 ± 0·1	5·3 ± 0·5	24·8 ± 0·8
Ratio of the area under the concentration–time curve for skin to that for plasma	13·1	36·3	29·3	26·4	26·7

The dose of salicylic acid was 25 μmol. The concentration of salicylic acid in the skin was determined by microdialysis and from its percent recovery (4·84%). Each value is the mean ± s.e.m. of results from three or four trials.

Table 4. Comparison of topical and transdermal delivery of salicylic acid from different ointments for rat skin with and without stratum corneum.

	Solbase	Poloid	White petrolatum	Hydrophilic poloid	Absorptive ointment
Ratio of maximum skin concentration for stripped skin to that in intact skin	46·2	1·6	1·0	2·0	0·8
Ratio of the area under the skin concentration–time curve between 0 and 4 h for stripped skin to that for intact skin	45·6	1·4	1·1	2·5	0·8
Ratio of maximum plasma concentration for stripped skin to that for intact skin	19·8	1·1	1·7	1·0	1·9
Ratio of the area under the plasma concentration–time curve between 0 and 4 h for stripped skin to that for intact skin	26·2	1·2	1·7	1·2	2·3

for evaluating the characteristics of liposomal delivery, because the concentration ratio or AUC ratio of a drug between skin tissues and plasma varies substantially. The skin is multi-layered in structure and comprises many histological layers such as the stratum corneum, the epidermis, the dermis and the hypodermis. Topical or transdermal delivery of drugs from the skin surface is known to be a process of passive diffusion, and a concentration gradient of the drug is formed within the skin tissues (Guy et al 1987). Therefore, in the evaluation of topical or transdermal delivery of a drug by skin microdialysis the position (distance from the skin surface) of the microdialysis probe implanted in the skin will be an important factor. When salicylic acid was administered intravenously, the concentrations of salicylic acid in the skin tissue were almost identical irrespective of the position of microdialysis probe. In contrast, after topical application of salicylic acid ointment the concentration of salicylic acid in the dialysate varied substantially depending on the depth of implantation of the microdialysis probe, because of the concentration gradient formed in the skin (data not shown). In particular, when the microdialysis probe was implanted deeply into the dermis or hypodermis the estimated concentration of salicylic acid in the skin tissue was almost identical with that in the plasma. In this study, therefore, special attention was paid to inserting the probe as superficially as possible.

When salicylic acid ointments were applied topically to skin with the stratum corneum intact the concentration of salicylic acid in the plasma increased in proportion to time (for example, r^2 was 0·9680 for solbase, 0·9450 for poloid and 0·9781 for absorptive ointment for the period from 0 to 2 h), indicating that the steady-state flux of salicylic acid through the stratum corneum could be expressed by a diffusion model for simple zero-order flux under Fickian conditions. On the other hand, in the stripped skin, a linear relationship was obtained between plasma concentrations of salicylic acid and the square root of time (r^2 was 0·9650 for solbase, 0·9323 for poloid and 0·9471 for absorptive ointment for the period from 0 to 2 h). These findings imply that the release of salicylic acid from these ointments is basically explained by matrix-type diffusion as expressed by Higuchi's equation.

By comparing AUC_{skin} with AUC_{plasma} for salicylic acid in intact skin and skin without the stratum corneum, delivery of salicylic acid was found to vary characteristically for the different ointments. For example, for intact skin, but not for skin without the stratum corneum, the ratio AUC_{skin}/AUC_{plasma} differed substantially for the different ointments (Tables 2 and 3). This variation of AUC_{skin}/AUC_{plasma} ratios could be because the ointment base forms a depot or reservoir in the stratum corneum. Also, the variation of the magnitudes of AUC_{skin} for skin without the stratum corneum could be because of differences between the release of salicylic acid from the ointments to the aqueous phase, and because the thermodynamic activity of salicylic acid differs in the different ointments. However, differences in thermodynamic activity can be discounted, because crystals were observed by optical microscopy in all preparations (400×), indicating that all the ointments were saturated with salicylic acid (Barry 1989). On the basis of these findings, each ointment will be characterized in relation to the role of the stratum corneum as follows:

The stratum corneum does not work as a barrier for absorptive ointment, because AUC_{skin} was no different for skin with and without the stratum corneum. Also absorptive ointment increases the retention of salicylic acid in the stratum corneum, because the ratio of AUC_{skin}/AUC_{plasma} was greatest for different ointment bases with intact skin (Table 2); because the stratum corneum had no

effect when white petrolatum was used, it might not function as a barrier with this ointment (Table 3 and 4). The low AUC_{skin} value for skin without the stratum corneum is because of the low rate of release of salicylic acid from the base, indicating that the release of salicylic acid from the ointment is the rate-determining step.

With solbase the stratum corneum functioned as a strong barrier, although salicylic acid is rapidly released from the base if water is present on the skin surface (Figure 1). Thus, on damaged skin salicylic acid will be delivered extensively to the skin tissue and central circulation from this ointment.

When the poloid and hydrophilic poloid bases were used removal of the stratum corneum had little effect, in contrast with the result for solbase (Tables 3 and 4). This indicates that the release of salicylic acid from poloid and hydrophilic poloid ointments is the rate-determining step; this is also true for white petrolatum. Hydrophilic poloid is a w/o-type emulsion lacking water, but its properties are considered to be similar to those of oleaginous bases such as white petrolatum, and poloid if water was not present in a sufficient amount to emulsify it.

As demonstrated in this study, the effect of different types of ointment base on the topical and transdermal delivery of salicylic acid was well characterized by determining concentrations of salicylic acid in skin tissue and in plasma.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research, No. 09672203, from the Ministry of Education, Science, Sport, and Culture (Japan).

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