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Enhanced penetration of mitomycin C through hairless mouse and rat skin by enhancers with terpene moieties

HIROKAZU OKAMOTO, MAKIKO OHYABU, MITSURU HASHIDA AND HITOSHI SEZAKI*

Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

The effects of four new percutaneous absorption enhancers containing an azacyclo ring and terpene chain (1-geranylazacycloheptan-2-one (GAH), 1-farnesylazacycloheptan-2-one (FAH), 1-geranylazacyclopentan-2,5-dione (GAPD), and 1-farnesylazacyclopentan-2-one (FAP)) and 1-dodecylazacycloheptan-2-one (Azone) on the percutaneous penetration of mitomycin C (MMC) through hairless mouse and rat skin in-vitro has been investigated. GAH, FAH, FAP and Azone enhanced MMC penetration by 20 to 60 times that of the control (ethanol). During the early part of the experiments, when the sink condition was maintained, FAH was the most effective for hairless mouse skin, whereas Azone showed the highest effect in the rat skin. The enhancing effect of GAPD was only about half that of the other enhancers, suggesting the importance of the polar group of the ring moiety in these compounds. The penetration of MMC through rat skin was also increased by pretreatment with these compounds, suggesting that the enhancers had a direct effect on the skin.

The topical application of antitumour agents for the treatment of diseases such as cutaneous cancer and psoriasis has many advantages in that by using this route of administration the systemic toxicity of the drug may be reduced. However, one of the problems in their topical application is the poor skin penetration of such compounds, e.g. mitomycin C (MMC).

1-Dodecylazacycloheptan-2-one (Azone) has recently received considerable attention as a percutaneous absorption enhancer for a wide range of drugs (Stoughton 1982, 1983). Azone has a structure which may be considered to be a chemical combination of pyrrolidone and decylmethylsulphoxide, both of which are known to be potent percutaneous absorption enhancers (Hadgraft 1984). Both the long alkyl chain moiety and the mild polar ring moiety of Azone seem to be necessary for its action as a penetration promoter. Examination of the effects of these moieties on the action of penetration enhancers, therefore, should give useful information on the development of absorption enhancers.

In the present study, terpenes, which are known to be endogenous components of skin, were chosen as the alkyl chain moieties, and four types of new percutaneous absorption enhancers with different azacyclo ring moieties were designed. The effect of these substances on the percutaneous penetration of MMC through the skin of hairless mouse and rat w investigated.

* Correspondence.

MATERIALS AND METHODS

Mitomycin C (MMC) was obtained from the Kyowa Hakko Kogyo Co., Japan. 1-Dodecylazacycloheptan-2-one (Azone) was kindly supplied by Nelson Research Center, USA. 1-Geranylazacycloheptan-2-one (GAH), 1-farnesylazacycloheptan-2-one (FAH), 1-geranylazacyclopentan-2,5-dione (GAPD) and 1-farnesylazacyclopentan-2-one (FAP) were synthesized by the Kuraray Co., Japan. The structures of these compounds are shown in Fig. 1. All other reagents used were of analytical grade.

Transdermal delivery rates of MMC were determined using an in-vitro diffusion cell procedure. The full-thickness dorsal skin of male hairless mice (7–9 weeks) was removed in one piece and adherent fat and other visceral debris was removed from the undersurface. Also, the full-thickness dorsal skin of male Wistar rats (230–250 g) was obtained after the removal of the hair, and adipose tissue was removed. The freshly excised skin was mounted in a diffusion cell with an available diffusion area of 8·04 cm^2 as described by Loftsson & Bodor (1981). The receptor compartment of each cell was filled with 48 mL of saline containing 100 ppm of kanamycin sulphate. Test formulations were prepared by suspending MMC in ethanol or in 3·3% ethanolic solutions of Azone, GAH, FAH, GAPD, or FAP to a total concentration of 10 mM. MMC suspension (1 mL) was applied to each donor compartment (donor volume, ca 19 mL). In all the experiments, the donor cell was sealed with a silicone stopper to prevent

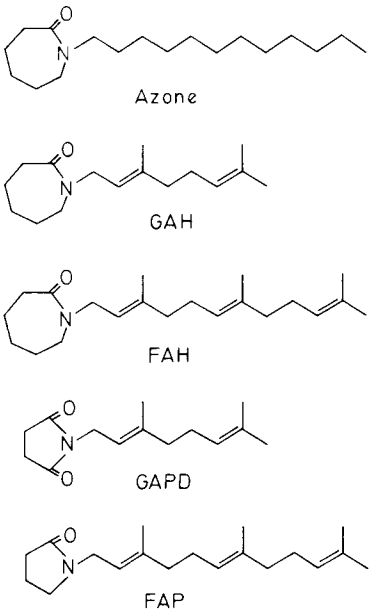

FIG. 1. The structures of Azone and the newly developed percutaneous absorption enhancers.

evaporation of the test sample. The diffusion cell was placed in a thermostatted chamber maintained at 37 °C and the receptor medium was stirred with a magnetic stirrer. At appropriate intervals, 1 mL of the receptor medium was withdrawn and this volume was replaced with fresh medium. Diffusion experiments were carried out for 16 h with hairless mouse skin and for 30 h with rat skin. At the end of each experiment, the drug in the donor phase was recovered with ethanol (25 mL).

In the pretreatment experiments, 1 mL of ethanol or 3·3% ethanolic solutions of Azone, GAH, FAH, GAPD, and FAP were applied to the donor side of the rat skin for 24 h. After removing the solution and washing the surface of the skin three times with 5 mL of ethanol, 2 mL aliquots of a 2 mM MMC solution in ethanol were applied and the diffusion experiments carried out as described above.

The concentration of MMC appearing in the receptor medium was measured by HPLC as described by Sasaki et al (1983). MMC recovered from the donor phase with ethanol was measured by spectrophotometric analysis at 360 nm after centrifugation and adequate dilution.

RESULTS

The structures of enhancers examined in this investigation are shown in Fig. 1. The geranyl chain has the *trans*-conformation. The farnesyl chain has the *trans*-conformation at the middle double bond and *cis*- and *trans*-conformation (3 : 7) at the double bond nearest to the ring moiety. All compounds are oils at room temperature and soluble in ethanol to make 3·3% solutions, but they are immiscible with water.

Fig. 2 shows the effects of Azone, GAH, FAH,

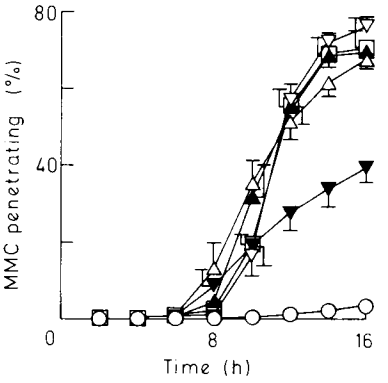

FIG. 2. The penetration of mitomycin C (MMC) through hairless mouse skin at 37 °C. One mL of MMC suspensions (total concentration, 10 mM) in ethanol (○) or in 3·3% ethanolic solutions of Azone (□), GAH (▽), FAH (△), GAPD (▼), or FAP (▲) was applied. Each point represents the mean value of three (Azone, GAH, FAH, GAPD, and FAP) or four (control) experiments and vertical bars indicate standard errors of the mean. Absence of a vertical bar means that the standard deviation is smaller than the size of a symbol.

GAPD, and FAP on the percutaneous absorption of MMC through hairless mouse skin in-vitro. The penetration of MMC without enhancer was low and only 1·23 and 3·53% of the total amount was recovered in the receptor compartment at 12 and 16 h, respectively. On the other hand, the penetration of MMC was greatly improved by the coexistence of Azone, GAH, FAH, and FAP and the amounts of MMC in the receptor phase were about 40 times higher at 12 h and 20 times higher at 16 h than the control in all cases. At 10 h, the highest penetration of 35·32 ± 10·93% of the applied dose in the receptor phase was produced by FAH, while FAP (31·54 ± 15·43%), Azone (19·51 ± 4·82%), and GAH (16·79 ± 9·25%) followed this. The value for FAH was significantly different ($P < 0·05$) from that for Azone and for GAH at this time. GAPD also enhanced the penetration of MMC but at 12 to 16 h its effect was about half that of the other enhancers.

Fig. 3 shows the permeation profiles of MMC through rat skin when applied with or without enhancers. Azone, GAH, FAH, and FAP markedly enhanced MMC penetration through rat skin. The

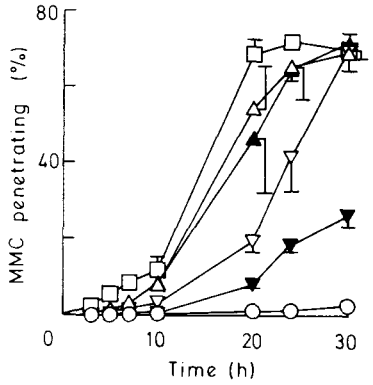

FIG. 3. The penetration of mitomycin C (MMC) through rat skin at 37 °C. One mL of MMC suspensions (total concentration, 10 mM) in ethanol (○) or in 3·3% ethanolic solutions of Azone (□), GAH (▽), FAH (△), GAPD (▼), or FAP (▲) were applied. Each point represents the mean value of three (GAH and GAPD) or four (control, Azone, FAH, and FAP) experiments and vertical bars indicate standard errors of the mean. Absence of vertical bar means that standard deviation is smaller than the size of a symbol.

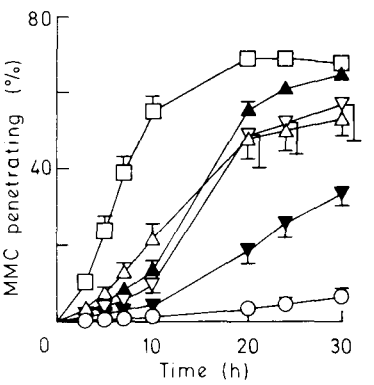

FIG. 4. The penetration of mitomycin C (MMC) through rat skin at 37 °C. The skins were pretreated with 1 mL of ethanol (○) or 3·3% ethanolic solutions of Azone (□), GAH (▽), FAH (△), GAPD (▼), or FAP (▲). After 24 h treatment, each skin was washed with ethanol and 2 mL of 2 mM MMC solution in ethanol was applied. Each point represents the mean value of three (GAH) or four (control, Azone, FAH, GAPD, and FAP) experiments and vertical bars indicate standard errors of the mean. Absence of vertical bar means that standard deviation is smaller than the size of a symbol.

enhancers with a geranyl chain (GAH and GAPD) showed a longer lag time than the others. After the prolonged lag time, GAH produced almost the same penetration rate as the other enhancers, although the amount of penetrated MMC was only equivalent to that of the other enhancers at 30 h. GAPD showed a less obvious effect. In the case of rat skin, Azone produced enhanced penetration of MMC compared with FAH at 3, 5, and 7 h, with GAH at 3, 5, 7, 10, 20, and 24 h, and with FAP at 3, 5, 7 and 24 h (at least $P < 0{\cdot}05$ in all comparisons).

In the above two series of experiments, the initial thermodynamic activity of MMC in the donor phase was kept constant by employing suspension formulations, and it is suggested that the increase in MMC penetration resulted from direct interactions of the enhancers with the skin. To elucidate this problem further, the effects of the enhancers were examined with pretreatment experiments and the results are shown in Fig. 4. Pretreatment of rat skin with Azone resulted in a rapid increase in MMC penetration after the application of MMC solution. On the other hand, appearance of MMC in the receptor phase was delayed after pretreatment with GAH, FAH, and FAP compared with pretreatment with Azone. GAPD also showed the smallest effect in this experimental system.

In the hairless mouse skin experiment, the amount of MMC remaining in the donor phase at the end of the experiment was 84·9% for control, while enhancers reduced this amount to 2–5% except for GAPD (36·3%). In the rat skin experiment, the control value was 77·0%, which was reduced to about 1–2% by enhancers except for GAPD (26·5%). The control value for the pretreatment experiment with rat skin was 81·7% and this was reduced to 3·1% by Azone and to about 10–17% by the other enhancers except for GAPD (43·1%). In all diffusion experiments, the amount of MMC disappearing from the donor phase correlated well with that appearing in the receptor phase.

DISCUSSION

Three approaches may be used to improve the percutaneous penetration of a drug; (i) the selection of a vehicle to increase drug release or change the hydration state of the skin, (ii) the modification of drug molecules to give them higher affinity for skin, and (iii) the use of percutaneous absorption enhancers to reduce the barrier function of the skin. In previous studies we reported the enhanced delivery of MMC through hairless mouse and rat skin by means of chemical modification of MMC (Hashida et al 1985; Mukai et al 1985). Among various prodrugs of MMC, the most successful result was obtained with 1a-*N*-benzyloxycarbonylmitomycin C which penetrated the skin 3·5 times faster than did MMC and which was fully converted to MMC in the skin.

In the present study, Azone, GAH, FAH, and FAP enhanced MMC penetration, about 40 times that of the control at 12 h in the experiment with

hairless mouse skin and about 20 to 60 times that of the control at 20 h with rat skin. Thus, the use of penetration enhancers appeared to be more advantageous than the prodrug approach for the model compound, MMC.

FAH and FAP showed approximately equal enhancement effects on the penetration of MMC through hairless mouse and rat skin. The variation in the size of the ring moiety in these molecules appeared to have had little effect on their action as penetration enhancers. GAH, however, was approximately twice as effective as GAPD in enhancing MMC penetration, indicating that the polar group of the ring moiety has an important effect on the potency of these types of penetration enhancers.

The rate of permeation of the skin barrier is directly related to the thermodynamic activity of the drug above the barrier. This activity is usually maintained at the highest level as long as any solid material remains in the applied material, i.e. in suspension (Higuchi 1978). The penetration profiles in the later period indicate that the maximum thermodynamic activity in the donor phase and sink condition in the receptor phase were not maintained beyond this point. An apparent equilibration in MMC concentration appeared to be reached between the donor phase, the skin, and the receptor phase at the end of the diffusion experiments using Azone, GAH, FAH, and FAP. Consequently, it is necessary to compare the enhancing effects of these four compounds in the early period, when the maximum thermodynamic activity condition of MMC in the donor phase and sink condition in the receptor phase were being maintained. The results show that FAH produced greater penetration of MMC than did Azone or GAH in hairless mouse skin at 10 h (Fig. 2), whereas Azone was more effective for rat skin (Fig. 3). The effectiveness of Azone in rat skin was also demonstrated in the pretreatment experiments (Fig. 4). These results suggest that the effects of these enhancers might vary depending on the species of animal skin to which they are applied. The site of action of Azone has been reported to be within the stratum corneum (Sugibayashi et al 1985). The difference in the effects of Azone and the other enhancers studied might result from the different affinity of these compounds for the stratum corneum. Variation in lipid and protein composition between both animal species may play a role in this problem.

The difference between the initially applied amount of MMC and the amount recovered (sum of that in the donor and receptor phase) should correspond to the amount of MMC existing in the skin at the end of the experiment or that of MMC degraded during the diffusion experiment. These differences of control systems were 11·6% of the dose (hairless mouse) and 20·4% (rat), whereas those of systems with enhancers ranged over approximately 21·2–27·5% (hairless mouse) and 26·5–29·2% (rat) except for GAPD (47·5%). Also in the pretreatment experiment with rat skin, the control value was 11·9% and enhancers increased this to 23·3–33·9%. These results mean that the enhancers may increase the amount of MMC in the skin.

From the results obtained in this investigation, it is concluded that some newly designed percutaneous absorption enhancers containing terpene moieties, i.e. GAH, FAH, and FAP, have the potential to enhance the percutaneous penetration of MMC, and are almost equivalent to Azone in this action.

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