Effect of 1-Alkyl- or 1-Alkenylazacycloalkanone Derivatives on Penetration of Mitomycin C through Rat Skin

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The effects of five new compounds containing an azacyclo ring and a terpene or an alkyl chain, i.e., 1-geranylazacyclohexan-2-one (6GU), 1-(3,7-dimethyloctyl)azacycloheptan-2-one (7GS), 1-(3,7,11-trimethyldodecyl)azacycloheptan-2-one (7FS), 1-geranylgeranylazacycloheptan-2-one (7GGU), and 1-geranylgeranylazacyclohexan-2-one (6GGU), on percutaneous penetration of mitomycin C (MMC) through rat skin in vitro were investigated in comparison with those of 1-farnesylazacycloheptan-2-one (7FU) and 1-dodecylazacycloheptan-2-one (Azone). In an in vitro diffusion experiment, 6GU, 7GS, 7FU, 7FS, 7GGU, 6GGU, and Azone significantly enhanced MMC penetration through rat skin compared with the control. The size of the azacyclo ring had little effect on the potency of these penetration enhancers. On the other hand, the changes in the length of the hydrophobic chain resulted in some variation in the activity of these compounds. Azone and 7FU, which have a hydrophobic chain of length of twelve carbons, were the most effective among enhancers with an azacycloheptanone ring, and 7GGU, with the longest hydrophobic chain, showed the weakest MMC penetration-enhancing activity. Among the compounds with an azacyclohexanone ring, 6GGU (with a longer hydrophobic chain) was more effective than 6GU (with a shorter hydrophobic chain). Saturation of the double bonds of the farnesyl group of 7FU decreased the enhancing ability.

Keywords—percutaneous absorption; percutaneous penetration enhancer; 1-dodecylazacycloheptan-2-one (Azone); 1-alkenylazacycloalkanone derivative; mitomycin C; terpene

The topical application of antitumor agents for the treatment of diseases such as cutaneous cancer and psoriasis has many advantages; in particular, the systemic toxicity of the drug may be reduced. However, one of the problems in topical application of antitumor agents is the poor skin penetration of such compounds, e.g. mitomycin C (MMC).

One approach to improve the low skin penetrability of a drug is the use of a penetration enhancer to reduce the barrier function of the skin. Some compounds such as 2-pyrrolidone and decyl methyl sulfoxide have been reported as potential percutaneous penetration enhancers, and recently 1-dodecylazacycloheptan-2-one (Azone) has received considerable attention as a percutaneous absorption enhancer for a wide range of drugs.1−3) 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.

In the previous report, four types of percutaneous absorption enhancers with azacyclo ring moieties and terpene chains were designed. Some of these substances improved the percutaneous penetration of MMC through the skin of hairless mouse and rat, and were almost equipotent to Azone in this regard.4) In the present study, five new compounds with different structural characteristics were designed and their effects on the percutaneous penetration of MMC through the rat skin were investigated.
Experimental

Materials—Mitomycin C (MMC) was obtained from Kyowa Hakko Kogyo Co., Japan. 1-Dodecylazacycloheptan-2-one (Azone) was kindly supplied by Nelson Research Center, U.S.A. 1-(3,7-dimethyloctyl)azacycloheptan-2-one (6GU), 1-farnesylazacycloheptan-2-one (7FU), 1-(3,7,11-trimethylundecyl)azacycloheptan-2-one (7FS), 1-geranylgeranylazacycloheptan-2-one (7GGU), 1-geranylgeranylazacyclohexan-2-one (6GGU) were synthesized by Kuraray Co., Japan. All other reagents used were of analytical grade.

Percutaneous Penetration Experiments—Transdermal delivery rates of MMC were determined in the same manner as described in the previous report.4) The full thickness dorsal skin of male Wistar rats (230–250 g) was obtained after the removal of the hair with electric clippers, followed by the careful removal of adipose tissue. The freshly excised skin was mounted in a sink-type diffusion cell with an available diffusion area of 8.04 cm² as described by Loftsson and Bodor.5) The receptor compartment of each cell was filled with 48 ml of saline containing 100 ppm of kanamycin sulfate. Test formulations were prepared by suspending MMC in ethanol with or without the compounds listed in Fig. 1. These compounds were contained at concentration of 3.3 v/v%. One milliliter of MMC suspension was applied to each donor compartment. In all the experiments, the donor cell was sealed with a silicone stopper to prevent evaporation of the test sample. The diffusion cell was placed in a thermostated 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 replaced with an equal volume of fresh medium. Diffusion experiments were carried out for 30 h. At the end of each experiment, the drug in the donor phase was recovered with ethanol (25 ml).

The concentration of MMC appearing in the receptor medium was measured by high-pressure liquid chromatography (HPLC) as described by Sasaki et al.6) MMC recovered from the donor phase with ethanol was measured by spectrophotometric analysis at 360 nm after centrifugation and adequate dilution.

Results and Discussion

The structures of enhancers examined in this investigation are shown in Fig. 1. Saturation of the side chain of 7FU yields the structure of 7FS, which can be considered as Azone methylated at three positions in its alkyl chain. Similarly, saturation of the side chain of 1-geranylazacycloheptan-2-one (7GU) (reported in previous paper) gives 7GS. All these
compounds are oils at room temperature.

Figure 2 shows the permeation profiles of MMC through rat skin when applied with or without the compounds listed in Fig. 1. The decrease in the amount of MMC in the receptor at 30 h in the system with Azone indicates that MMC may decompose in the receptor phase. The pH-rate profile for the decomposition of MMC was reported to show a minimum degradation rate at pH 7.5—8.0.7) We did not, however, follow the pH of the receptor saline during our experiment. A peak with shorter retention time than that of MMC was observed on a chromatogram. This peak was much smaller than that of MMC. The absorption spectra (240—420 nm) of the donor and receptor solutions were each recorded at the end of every experiment. A clear peak of MMC was recognized at 360 nm and in some cases a small peak or shoulder was observed at 280 nm. The decomposition of MMC in this study, if any, seems to be minor and should not result in misinterpretation of the penetration-enhancing activity.

6GU, 7GS, 7FU, 7FS, 7GGU, and 6GGU as well as Azone markedly enhanced MMC penetration through rat skin (p < 0.001 in all comparisons at 30 h). At 30 h after beginning the penetration experiment, Azone produced no significant difference in amount of MMC in the receptor compartment as compared with 6GU, 7GS, 7FU, 7FS, 7GGU, and 6GGU. Azone, however, enhanced the penetration of MMC when compared with 6GU and 7GGU at 24 h and compared with 6GU, 7FS, 7GGU, and 6GGU at 20 h (at least p <0.05 in all comparisons). 7GGU (with a geranylgeranyl chain) was less effective than Azone, 6GU, 7GS, 7FU, or 7FS.

The amount of MMC remaining in the donor phase at the end of the experiment (control value, 68.35%) was significantly decreased by these compounds (p < 0.001 in all comparisons). Azone decreased this amount (0.93%) significantly more than 7FS (4.07%), 7GGU (12.84%), or 6GGU (6.15%). 6GU (4.77%), 7GS (1.75%), and 7FU (1.21%) were not significantly different from Azone in this regard.

To examine the structure—activity relationship of these penetration enhancers, sets of two compounds were compared for ability to promote MMC penetration by means of the paired t-test. Data on 7GU, 7FU, 1-geranylazacyclopentan-2,5-dione (5GUDO), and 1-farnesylazacyclopentan-2-one (5FU) are cited from previous reports4,8) (represented by asterisks.

![Figure 2. The Penetration of MMC through Rat Skin at 37°C](image)
in the discussion below). The amounts of MMC in the receptor medium at 20, 24, and 30 h and that remaining in the donor phase at 30 h were compared (the criterion of statistical significance was set at $p < 0.05$). The consistency of the two series of experiments was warranted by the facts that no significant difference was observed in the results of comparisons of the control experiments, the effects of Azone and the effects of 7FU.

The size of the azacyclo ring had little effect on the potency of these penetration enhancers ($7GU^* = 6GU$, $7FU^* = 5FU^*$, $7GGU = 6GGU$). But the polar group of the ring moiety had an important effect ($7GU^* > 5GUDO^*$ at 20 and 30 h, $6GU > 5GUDO^*$ at 30 h), as reported previously.

The length of hydrophobic chain caused greater apparent variation in the activity of these compounds as penetration enhancers. Comparisons of the enhancers with an azacycloheptane ring showed that the closer to twelve carbons the length of the hydrophobic chain was (Azone and 7FU), the more effective the penetration enhancer was, and the compound with the longest hydrophobic chain (7GGU) showed the weakest MMC penetration-enhancing activity ($7GU^* > 7GGU$ at 30 h, $7FU > 7GGU$ at 20, 24, and 30 h, $7GS > 7FS$ at 30 h). In the compounds with an azacyclohexanone ring, on the other hand, the compound with a longer hydrophobic chain (6GGU) was more effective than that with a shorter hydrophobic chain (6GU) at 24 h.

In the alkyl methyl derivatives of sulfoxides, the optimum chain length was reported to be ten carbons.\textsuperscript{1} The balance of hydrophilicity and hydrophobicity in a molecule may have a major effect on the activity as a penetration enhancer. A different polar group may require a different hydrophobic group for maximum penetration enhancing activity.

6GU with a geranyl chain showed a longer lag time, as did $7GU^*$ and $5GUDO^*$ in a previous report, than the others. The enhancers with a geranyl chain seems to need a longer period to attain the steady state of penetration of MMC.

The effect of saturation of the hydrophobic chain was also examined. Saturation of the double bonds of the farnesyl group decreased the activity ($7FU > 7FS$ at 30 h), whereas saturation of the double bonds of the geranyl group had no significant effect ($7GU^* = 7GS$).

Through our two series of experiments, several 1-alkyl- or 1-alkenylazacycloalkanone derivatives were proved to be potent penetration enhancers for MMC. Further investigation is in progress in our laboratory to elucidate the mechanisms of action of these compounds.

References and Notes

8) 7GU, 7FU, 5GUDO, and 5FU were abbreviated as GAH, FAH, GAPD, and FAP, respectively, in previous reports.