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Enhanced delivery of mitomycin C derivatives through hairless mouse and rat skins

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The percutaneous permeation characteristics of two lipophilic mitomycin C derivatives with aromatic moieties were determined using excised hairless mouse and rat skins and compared with those of mitomycin C (MMC). 1a-*N*-Benzylmitomycin C penetrated more readily through both kinds of skin than MMC without metabolic conversion. 1a-*N*-Benzyloxycarbonylmitomycin C effected a 4-fold increase in the delivery of MMC through the rat skin and was completely converted to MMC by an enzyme in rat skin. However, saturation of the metabolic conversion activity was observed in the hairless mouse skin.

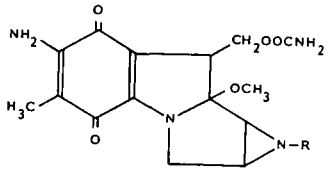
Transdermal delivery of anticancer agents seems to be a promising method of treating various diseases including epithelial neoplasms and psoriasis, because of the improvement in local bioavailability and the decrease in systemic exposure compared with systemic drug administration. However, some drugs have physicochemical characteristics such as low lipophilicity and high melting point (i.e., poor biphasic solubility) which are undesirable for dermal absorption. Mitomycin C (MMC) represents one class of such drugs and exhibits these properties.

The transdermal delivery of poorly absorbable drugs may be enhanced by the development of lipophilic and/or low-melting derivatives of them. Thus, various kinds of prodrugs of antimetabolic agents such as 5-fluorouracil (Møllgaard et al 1982) and 6-mercaptopurine (Sloan et al 1983) have been developed. In a previous investigation, we developed several lipophilic derivatives of MMC with aromatic moieties, such as 1a-*N*-benzylmitomycin C (BM) and 1a-*N*-benzyloxycarbonylmitomycin C (BOCM) (I) (Sasaki et al 1983a). They showed increased lipophilicity and whereas BM was active by itself, BOCM was only active after regeneration into the parent drug (Sasaki et al 1983a, b).

In the present study, the apparent permeability of potential derivatives of MMC was examined using excised hairless mouse and rat skins.

MATERIALS AND METHODS

Mitomycin C (MMC) was obtained from Kyowa Hakko Kogyo Co., Japan. 1a-*N*-Benzylmitomycin C (BM) and 1a-*N*-benzyloxycarbonylmitomycin C (BOCM) were synthesized as described by Sasaki et al (1983a). All other chemicals were of reagent grade and obtained commercially.



I. Chemical structures of mitomycin C derivatives tested. MMC: R = H; BM: R = CH_2-C_6H_5; BOCM: R = $COOCH_2C_6H_5$.

Transdermal delivery rates were determined using an in-vitro diffusion cell procedure. Male, hairless mice (12–15 weeks) were killed by cervical dislocation and the whole dorsal skin was removed in one piece. The full-thickness skin was mounted in a diffusion cell after removing adherent fat and other visceral debris from the undersurface. The skins of male, Wistar rats (320–380 g) were also used for permeability experiments. The hair was removed from anaesthetized rats using a hair clipper and the abdominal skin was excised. The diffusion cells used in the present experiment are the same as those used by Loftsson & Bodor (1981) and have an available diffusion area of 8·04 cm^2. Isotonic phosphate buffer (pH 7·4, 48 ml) containing kanamycin sulphate (100 ppm) was used as the receptor medium. Test formulations were prepared by suspending (MMC) or dissolving (BM and BOCM) to a total concentration of 2 mM in isopropyl myristate and 0·5 ml of each was applied. 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, samples of the receptor medium were withdrawn. After 24 h, the drug remaining in the donor medium was recovered with 25 ml of methanol. The results reported for each

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experiment were the average values from four replicate diffusion experiments using skins obtained from four animals.

The concentrations of applied drugs and regenerated MMC were measured by a high-pressure liquid chromatography (HPLC) as described by Sasaki et al (1983a). The concentration of drug recovered in the methanol solution was measured, after centrifugation, by spectrophotometric analysis at 355–360 nm.

RESULTS

The physicochemical properties of the test compounds studied are summarized in Table 1.

Fig. 1 shows the comparative cumulative permeation profiles of MMC through hairless mouse and rat skins. The accumulation of MMC in the receptor medium increased linearly after a short lag time in both experiments. The transference of MMC was faster through the hairless mouse skin than through the rat skin.

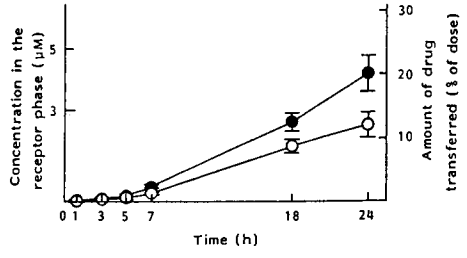

FIG. 1. Permeability of mitomycin C through hairless mouse (●) and rat (○) skins as concentration (left ordinate) and percentage of drug applied (right ordinate) in the receptor phase. Vertical bars indicate s.d. and each point is the mean of four experiments.

Table 1. Physicochemical properties of compounds tested.

Compound	Mp (°C)	Partition coeff (Octanol–water)	Solubility (mM) Water (25 °C)	n-Hexane (25 °C) (×10³)	Isopropyl myristate (37 °C)
MMC	>270	0·41	2·73	0·023	0·019
BM	119–121	38·6	1·49	0·726	5·67
BOCM	102–104	113·0	0·52	0·471	3·28

Table 2. Amount of drug in the donor and receptor mediums 24 h after the start of the diffusion experiment.

Compound	Hairless mouse skin Donor medium	Hairless mouse skin Receptor medium	Rat skin Donor medium	Rat skin Receptor medium
MMC (I)	55·9 ± 2·5	20·1 ± 3·1	82·4 ± 8·9	12·2 ± 1·7
BM (II)	18·4 ± 5·5	37·5 ± 4·3	34·5 ± 5·9	24·2 ± 6·4
BOCM (III)	34·5 ± 1·5	22·0 ± 1·7 (I) 13·9 ± 2·6 (III)	39·7 ± 13·7	46·0 ± 8·0 (I) 0 (III)

Results are expressed in terms of percent of the applied dose. The mean values of four diffusion experiments are given with the standard deviations.

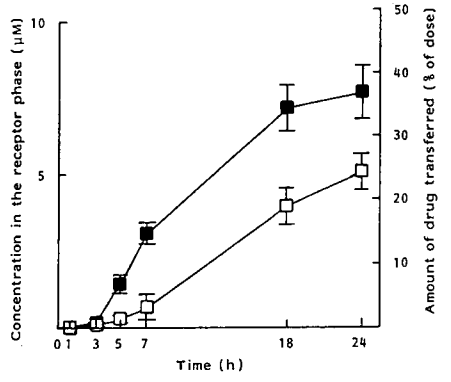

FIG. 2. Permeability of 1a-*N*-benzylmitomycin C through hairless mouse (■) and rat (□) skins as concentration (left ordinate) and percentage of drug applied (right ordinate) in the receptor phase. Vertical bars indicate s.d. and each point is the mean of four experiments.

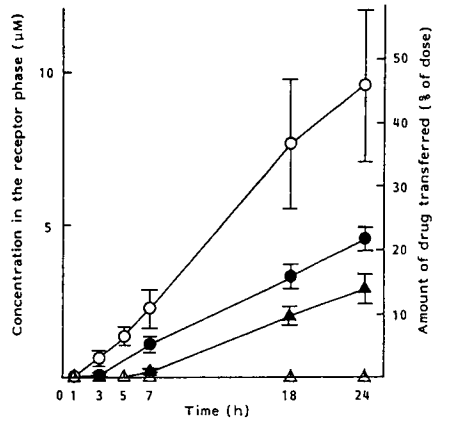

FIG. 3. Permeability of 1a-*N*- benzyloxycarbonylmitomycin C through hairless mouse (closed symbols) and rat (open symbols) skins as concentration (left ordinate) and percentage of drug applied (right ordinate) in the receptor phase. ● and ○, MMC from applied BOCM. ▲ and △, BOCM from applied BOCM. Vertical bars indicate s.d. and each point is the mean of four experiments.

Fig. 2 shows the permeation profiles of BM which also transferred more rapidly through the hairless mouse skin than the rat skin. During the course of experiment, MMC was not detected in the receptor medium in any case.

The transdermal delivery of BOCM is illustrated in Fig. 3. When BOCM was applied to the hairless mouse skin in isopropyl myristate solution, MMC and BM were detected in the receptor medium after a short lag time. The transference rate of MMC into the receptor chamber was about 1·5 times faster than that of BOCM. However, only MMC was detected in the receptor medium when BOCM was applied to the rat skin.

The amounts of drugs in the donor and receptor mediums 24 h after the start of the diffusion experiments are summarized in Table 2. MMC and BM showed greater transference through the hairless mouse skin than through the rat skin. The amount of BM and BOCM which were transferred through the rat skin within 24 h was three to four times higher than that of MMC.

DISCUSSION

The principal barrier to percutaneous transport is the stratum corneum and the major processes determining transport through this are partitioning and diffusion. These are directly affected by the molecular characteristics of the penetrants, such as solubility, size and shape.

As expected, the introduction of aromatic moieties to MMC as the 1a-*N* position resulted in changes in its physicochemical properties. BM and BOCM showed fairly low melting points and high solubilities in n-hexane compared with MMC, suggesting the increase of thermodynamic activity in their pure solid state (Higuchi 1982). The lipophilicities of BM and BOCM were shown to be much higher than that of MMC, e.g. the n-octanol–water partition coefficient increased from 0·41 (MMC) to 38·6 (BM) and 113·0 (BOCM).

The present results clearly demonstrate that the transdermal delivery of MMC was markedly improved by the chemical modification of its molecular structure. BM showed enhanced delivery to almost the same extent in both the mouse and rat experiments while BOCM was more effective in the rat skin.

In the permeation experiment, BM transferred into the receptor medium keeping its original structure. This result suggests that BM does not undergo any metabolic conversion in the mouse or rat skins, as is the case in plasma and in liver homogenates (Sasaki et al 1983b). On the other hand, MMC was detected in the receptor medium as a major conversion product after the application of BOCM. Since BOCM is stable in a pH 7·4 buffer solution (Sasaki et al 1983b), the conversion of BOCM to MMC was considered to occur by metabolic cleavage of the carbamate linkage in the skin. Thus BOCM could be acting as a potential prodrug of MMC in the present system. In the mouse experiment, BOCM appeared in the receptor medium as well as MMC, suggesting a limitation of the metabolic capacity in mouse skin. The relatively low efficiency of BOCM in increasing the permeability of MMC in the mouse skin may be explained by this low enzymatic activity.

Except in the case of BOCM, which was affected by the metabolic activity, the hairless mouse skin appeared to be more permeable or to have lower diffusion resistance than the rat skin because MMC and BM penetrated it about 1·7 times faster. This result is in good agreement with those obtained for various penetrants (Bronaugh et al 1982).

On the basis of the present results, it was concluded that the chemical modification of the molecular structure of MMC to more lipophilic and low-melting compounds would offer a promising method of achieving its optimal transdermal delivery. Additional improvement of absorption can be attained by increasing the drug concentration in the vehicle to its maximum solubility.

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