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Enhanced delivery of mitomycin C prodrugs through the skin

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Summary

The percutaneous permeation characteristics through the excised rat skin of seven lipophilic 1a-N-substituted derivatives of mitomycin C (I) were determined in relation to their physicochemical and pharmaceutical properties. Among the four derivatives of compound I [benzyl (II), benzoyl (III), benzylcarbonyl (IV) and benzoyloxycarbonylmitomycin C (V)] possessing aromatic pro-moieties with different linkage structures, compounds II and V showed higher steady-state penetration rates than I. II penetrated the skin without metabolic conversion, while V was completely converted to I. Three alkoxycarbonyl-type derivates of I [propyl (VI), pentyl (VII), and nonyloxycarbonylmitomycin C (VIII)] with different alcoholic pro-moieties were studied to elucidate the effect of the carrier moiety on their permeation. VII showed an improved permeability, but the most lipophilic prodrug, VIII, failed to enhance the delivery of I. Compounds III and VI, which had high melting points and low biphasic solubilities, only slightly permeated the skin. Permeation rates of the test compounds reached a plateau in the concentration range over their solubilities in the vehicle; the maximum rates of II, V, and VII were, respectively, 6.5, 5.3, and 3.4 times larger than that of I.

Introduction

Topical application of anticancer agents may be advantageous for the treatment of diseases such as cutaneous cancer and psoriasis, since the systemic toxicity of such

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agents can be either circumvented or at least minimized by this administration modality. However, most agents are generally ineffective when applied topically, owing to physicochemical characteristics that interfere with dermal absorption; i.e. they may be high melting and/or polar lipophobic molecules that hinder their facile absorption through the skin (Higuchi, 1960). A promising approach to improving the transdermal delivery of such drugs may be the development of lipophilic and low-melting products that revert to the parent compound by virtue of enzymatic or chemical lability in the skin. Based on these considerations, prodrugs of antimetabolic agents including vidarabine (Fox et al., 1979; Yu et al., 1979), 5-fluorouracil (Møllgaard et al., 1982), and 6-thiopurines (Sloan et al., 1983) have been developed.

Mitomycin C (I), an antitumor antibiotic, represents one class of drugs that shows poor transdermal delivery. In a previous investigation, we synthesized several 1a-N-substituted derivatives of I, such as benzyl (II), benzoyl (III), benzylcarbonyl (IV), and benzyloxycarbonylmitomycin C (V) (Table 1), which possess aromatic pro-moieties through different linkage structures (Sasaki et al., 1983b). Biological tests revealed that they themselves exhibited characteristic antitumor activities (II), or after being regenerated to the parent drug (III, IV and V) (Sasaki et al., 1983b and c). In addition, several alkoxycarbonyl-type prodrugs with different leaving moieties, such as propyloxycarbonyl (VI), pentyloxycarbonyl (VII), and nonyloxycarbonylmitomycin C (VIII) (Table 1), had been synthesized following the discovery that the carbamate linkage has the most suitable characteristics regarding chemical stability and biological lability (Sasaki et al., 1983a). These products showed increased lipophilicity with an increase of the alkyl chain length, and exhibited antitumor activity similar to that of compound I.

In the present study, the apparent permeability of these prodrugs through the excised rat skin was examined in relation to their physicochemical and pharmaceutical properties.

Materials and Methods

Materials

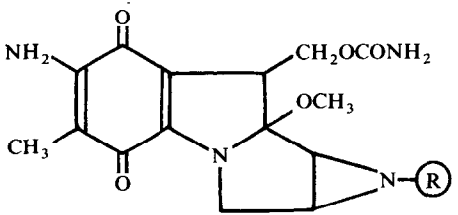
Compound I was obtained from Kyowa Hakko Kogyo. Compounds II–VIII were synthesized as described previously (Sasaki et al., 1983a and b). All other chemicals were of reagent grade and obtained commercially from Nakarai Chemicals.

In vitro permeation experiment using the rat skin

Transdermal delivery rates were determined using an in vitro diffusion cell procedure. Full-thickness abdominal skins of male Wistar rats weighing 320–380 g were used. The hair of the anesthetized rat was removed with electric hair clippers and the abdominal skin was excised after careful shaving. The skin was mounted in a diffusion cell after removing adherent fat and other visceral debris from the undersurface. The diffusion cell was a similar type used by Loftsson and Bodor (1981) and had an available diffusion area of 8.04 cm^2. Isotonic phosphate buffer (pH 7.4, 48 ml) containing kanamycin sulfate (100 ppm) was used as the receptor

TABLE 1

STRUCTURES AND PHYSICOCHEMICAL PROPERTIES OF MITOMYCIN C PRODRUGS



Compound	R	m.p. (°C)	PC [a]	Solubility (mM)		
				Water (25°C)	Isopropyl myristate (37°C)	*n*-Hexane ($\times 10^3$) (25°C)
I Mitomycin C (MMC)	-H	> 270	0.41	2.73	0.019 [b]	0.023 [b]
II Benzyl-MMC	$-CH_2-C_6H_5$	119–121	38.6 [b]	1.49 [b]	5.67 [b]	0.726 [b]
III Benzoyl-MMC	$-CO-C_6H_5$	> 270	77.9	0.01	0.009 [b]	< 0.020 [b]
IV Benzylcarbonyl-MMC	$-COCH_2-C_6H_5$	154–156	34.5 [b]	2.24 [b]	0.41	0.209 [b]
V Benzyloxycarbonyl-MMC	$-COOCH_2-C_6H_5$	102–104	113.0 [b]	0.52 [b]	3.28 [b]	0.415
VI Propyloxycarbonyl-MMC	$-COOC_3H_7$	203–207	32.7	0.33	0.11	< 0.020
VII Pentyloxycarbonyl-MMC	$-COOC_5H_{11}$	89–93	279.2	0.59	8.22	2.02
VIII Nonyloxycarbonyl-MMC	$-COOC_9H_{19}$	139–141	3673	0.00025	12.62	0.304

[a] Partition coefficient between *n*-octanol and water.

[b] These values are update and, thus different, from those reported previously (Sasaki et al., 1983b).

medium. Test formulations were prepared by dissolving or suspending the drugs in isopropyl myristate and generally applied in the amount of 0.5 ml. The diffusion cell was placed in a thermostated chamber maintained at 37°C and the receptor phase was stirred by a magnetic stirrer. At appropriate intervals, samples of the receptor fluid were withdrawn. After 24 h, the drug in the donor phase was recovered with 25 ml of methanol. The results represent the mean values from four replicate diffusion experiments.

Analytical methods

The concentrations of drugs in the receptor phase were measured by high-pressure liquid chromatography (HPLC) as described previously (Sasaki et al., 1983a and b). The stationary phase consisted of Cosmosil $5C_{18}$ packed column (4.6 × 150 mm, Nakarai Chemicals); a short guard column packed with Lichrosorb RP-2 (E. Merck) was used to guard the main column. Mixtures of methanol–water were used as the mobile phase at a flow rate of 0.8 ml/min. The standard solutions were chromatographed and calibration lines were constructed on the basis of peak-area measurements. The concentration of drug in the methanol solution recovered from the donor phase was measured by spectrophotometric analysis after centrifugation.

Determination of partition coefficient and solubility

Partition coefficients of compounds I–VIII were determined in an *n*-octanol (2 ml)–distilled water (8 ml) system at 25°C at a total drug concentration of 0.01 mM. The solubilities in distilled water, isopropyl myristate and *n*-hexane were determined by suspending an excess amount of the compound in the solvent (3 ml) for 48 h with agitation, followed by filtration and analysis. Experiments were carried out in quadruplicate and concentrations were determined by HPLC. These procedures afforded more precise data than methods reported earlier (Sasaki et al., 1983b).

Results

Comparison of penetration rates through the skin

In order to compare the penetration characteristics of I and its prodrugs, permeation experiments were carried out at a fixed donor phase concentration of 2 mM. Drugs were applied in the form of a suspension (I, III, IV and VI) or a solution (II, V, VII and VIII) (Fig. 1).

When I suspended in isopropyl myristate was applied on the surface of the rat skin, it appeared in the receptor phase after a short lag time. After 24 h, the amount of I in the receptor phase reached 12.2% of the applied dose. II was transferred through the skin more rapidly than I, with 24.2% of the dose being recovered after 24 h. However, I was not detected in the receptor phase. Compounds III and IV had only a negligible effect on the transfer of I. Among four derivatives possessing aromatic pro-moieties, only V showed an increased delivery of I through the skin. After 24 h, the amount of I in the receptor phase reached 46.0% of the applied dose, but no V was detected.

TABLE 2

PENETRATION RATES AND DONOR-PHASE REMAINING PERCENT OF MITOMYCIN C PRODRUGS IN DIFFUSION EXPERIMENTS USING THE RAT SKIN

Compound	Initial donor concentration (mM)	Vehicle condition	Steady-state penetration rate (nmol/h)			Percent remaining in donor-phase after 24 h
			Parent drug : I	Prodrug	Total	
I	0.25 [a]	Susp.	8.59 ± 1.58 [b]			7.1 ± 3.9
	2.0	Susp.	6.10 ± 0.94			82.4 ± 8.9
	10.0	Susp.	8.99 ± 0.63			86.2 ± 3.2
II	2.0	Sol.	0	12.48 ± 3.07	12.48 ± 3.07	34.5 ± 5.9
	10.0	Susp.	0	58.58 ± 18.2	58.58 ± 18.2	59.0 ± 10.0
III	2.0	Susp.	0.01 ± 0.01	0.12 ± 0.03	0.13 ± 0.03	80.1 ± 2.6
IV	2.0	Susp.	3.21 ± 0.57	2.03 ± 0.49	5.24 ± 1.01	80.1 ± 7.6
V	0.5	Sol.	3.17 ± 0.26	0	3.17 ± 0.26	16.3 ± 5.2
	2.0	Sol.	21.15 ± 4.49	0	21.15 ± 4.49	39.7 ± 13.7
	3.3	Sol.	40.76 ± 5.17	0	40.76 ± 5.17	59.5 ± 5.5
	6.0	Susp.	37.63 ± 10.04	0	37.63 ± 10.04	65.7 ± 6.2
	10.0	Susp.	47.21 ± 14.84	0	47.21 ± 14.84	69.2 ± 15.7
VI	2.0	Susp.	2.11 ± 1.18	0.19 ± 0.09	2.20 ± 1.34	91.5 ± 3.08
VII	2.0	Sol.	9.19 ± 2.10	0.10 ± 0.05	9.29 ± 2.19	70.3 ± 6.67
	10.0	Susp.	30.71 ± 1.90	0.10 ± 0.05	30.81 ± 1.97	72.4 ± 4.0
VIII	2.0	Sol.	0.19 ± 0.11	0	0.19 ± 0.11	92.8 ± 2.38

[a] Donor phase volume was 1 ml. Except for this experiment, donor phase volume was 0.5 ml.
[b] Results are expressed as the mean ± S.D. of four experiments.

Among compounds VI–VIII, which have serial alcoholic pro-moieties through the carbamate linkage, VII showed enhanced delivery of I. The transfer of VI was less than that of I and only a slight amount of I was detected in the receptor phase at 24 h after the application of VIII.

From these results, steady-state penetration rates were calculated from the slopes of the amounts diffused versus time profiles at time intervals of 5–24 h. The slope was estimated by the least-squares method for each profile. The mean values of four experiments are summarized in Table 2. The amounts of drug recovered from the donor phase after 24 h diffusion experiments are also listed. The remaining amounts, i.e. the amounts of drug disappearing from the vehicle, correlated well with those appearing in the receptor phase.

Penetration rates at various drug concentrations

To determine the effect of drug concentration in the vehicle on the penetration rate, diffusion experiments were carried out at different drug concentrations and the results are listed in Table 2. Fig. 2 shows the relationship between the drug concentration in the vehicle and the steady-state penetration rates for I and V.

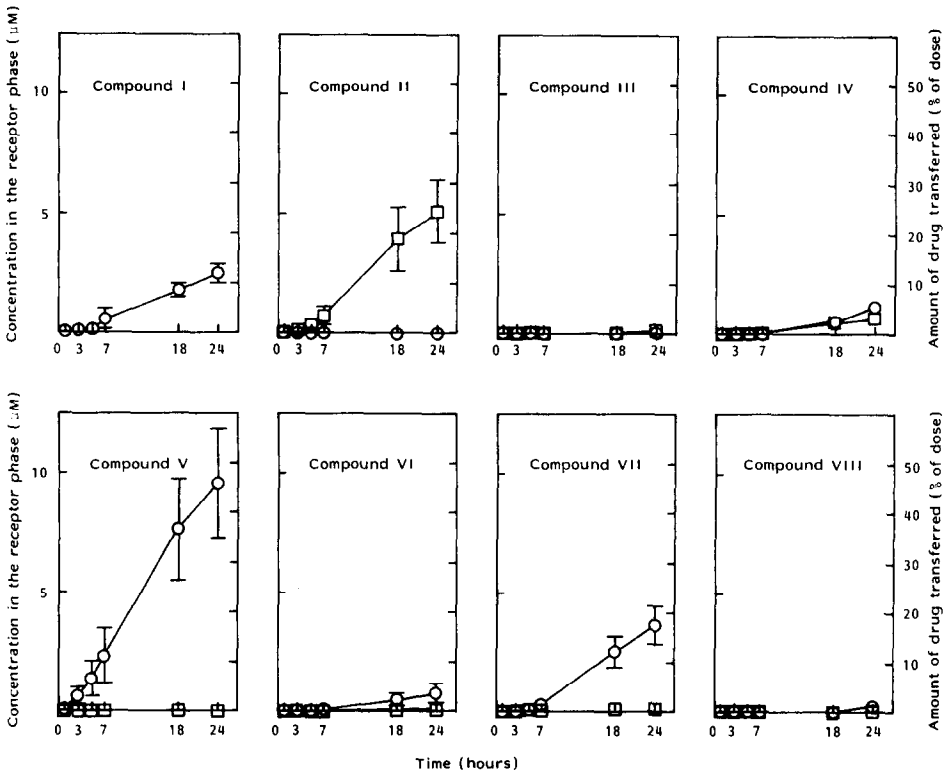


Fig. 1. Permeability of mitomycin C and its prodrugs through the rat skin as concentration (left ordinate) and percentage of drug applied (right ordinate) in the receptor phase. □, prodrug; ○, mitomycin C regenerated from prodrugs. Vertical bars indicate S.D. and each point is the mean of four experiments.

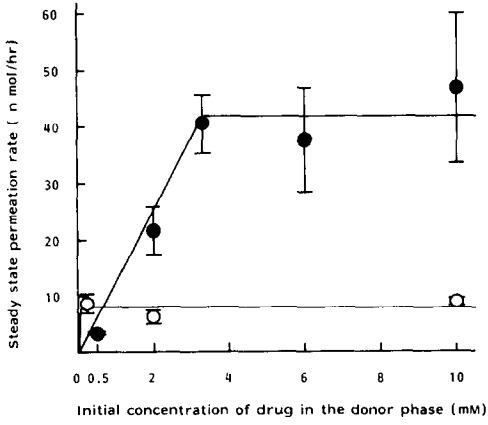


Fig. 2. Effect of the donor phase concentration on the permeability of mitomycin C and prodrug V through the rat skin. ○, mitomycin C; ●, prodrug V. Vertical bars indicate S.D. and each point is the mean values of four experiments.

I exhibited almost identical penetration rates at donor concentrations of 0.25, 2.0 and 10.0 mM; these values all exceeded the solubility of I in isopropyl myristate (0.02 mM). The penetration rates of V rose with an increase in the donor concentration until the latter reached the solubility of V (3.3 mM). Then V showed almost equal penetration rates to this value ($P > 0.2$) in the concentration range where V exists in the form of suspension (Table 2). At a donor phase concentration of 10 mM, compounds II, V and VII gave steady-state penetration rates that were 6.5, 5.3 and 3.4 times higher, respectively, than that of I.

Discussion

Drugs are transported across the skin barrier by diffusion through such tissue as hair follicles, sebaceous glands, and sweat glands, or by diffusion through the stratum corneum. For many drugs, the latter route is considered to be overwhelmingly dominant after the establishment of steady-state conditions. Therefore, the passive permeability properties of the stratum corneum constitute the major diffusional resistance of the skin (Idson, 1975).

In a simple diffusion-barrier system consisting of the stratum corneum, the driving force behind the drug movement is thought to be the difference in the thermodynamic potential between the vehicle and the deeper tissues (Higuchi, 1960). Conversely, the only significant factor concerning the vehicle appears to be the thermodynamic activity of the penetrating drug contained in it. The affinity of the drug substance for the stratum corneum is another determinant for the dermal penetration of a drug. Since these aspects of the drug are closely related to the agent's molecular properties such as shape, size, intra- and intermolecular binding, and solubility, the chemical manipulation of the molecular structure to a transit form (prodrug) with favorable properties offers a promising approach to improve its topical bioavailability (Higuchi, 1978). In the present paper, the apparent permeability and simultaneous metabolism of prodrugs of I were studied in relation to their molecular characteristics.

As expected, the introduction of leaving moieties at the 1a-N position resulted in remarkable changes in molecular characteristics of I (Table 1). Compounds II, IV, V, VII and VIII showed fairly low melting points and high solubilities in *n*-hexane compared with I, suggesting an increase in thermodynamic activities of their pure solid states (Higuchi, 1978). However, compounds III and VI showed considerably higher melting points and low biphasic solubilities which suggested that their pure forms are strongly internally bound so as to render them thermodynamically unavailable. The slight penetration of III and VI were considered to reflect these disadvantageous characteristics.

Substitution of the 1a-N position of I by lipophilic pro-moieties increased the lipophilicity of the prodrugs and therefore, generally, enhanced their dermal absorption. The partition coefficient of I (0.4) in an octanol/water system increased to values between 34.4 (IV) to 3673 (VIII). Among five compounds showing increased partition coefficients as well as low melting points, II, V and VII exhibited greater

penetration than I. VIII, with the longest alcoholic pro-moiety and extremely high lipophilicity, showed no penetration in the present system. This compound may be tightly bound to the vehicle or to the skin.

Acylated products of I such as III and IV were shown to be converted to I in the liver homogenate but not in other tissues including plasma (Sasaki et al., 1983c). Accordingly, compound I recovered in the receptor phase was considered to be produced in the receptor medium by the chemical hydrolysis of imide bond (Sasaki et al., 1983c).

In previous investigations, it was demonstrated that II underwent no metabolic conversion in any tissue medium but showed considerable antitumor activity in its own right; i.e. II appeared to be an effective analog of I (Sasaki et al., 1983b). These findings were in agreement with the present results; only II was detected in the receptor phase, unlike the other prodrugs. On the other hand, skin permeability studies indicated extensive metabolism of V and VII in the skin, which had been demonstrated to be stable in pH 7.4 buffer (Sasaki et al., 1983a and c).

Thus among seven derivatives of I, only V and VII exhibiting low melting points, high biphasic solubilities, high lipophilicities, and complete metabolic conversion to the parent drug succeeded in enhancing the transdermal delivery of I. The maximum penetration rates of I after application of V and VII, determined at the maximum thermodynamic condition (10 mM), were 5.3 and 3.4 times faster than that of I, respectively. Although similar results were obtained in experiments using the hairless mouse (unpublished data), species differences in metabolic capacity may exist.

From the present results, we conclude that chemical modification of the molecular structure of I to low-melting and more lipophilic compounds offers a promising method for achieving optimal transdermal delivery of I. Comparative analysis of the present findings with those obtained for different kinds of prodrugs of I such as dextran conjugate (Kojima et al., 1980; Takakura et al., 1984), agarose bead conjugate (Kojima et al., 1978), and polyamino acid conjugate (Roos et al., 1984) may provide additional information useful for further drug design of I.

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