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Further Evaluation of a New Penetration Enhancer, HPE-101

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Abstract—The penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101), significantly enhanced the excretion of topically applied [^{14}C]indomethacin when dissolved in dipropylene glycol, triethylene glycol, diethylene glycol, 1,3-butylene glycol, trimethylene glycol, glycerin, water, silicone or triethanolamine, but not when dissolved in ethanol, isopropyl alcohol, oleyl alcohol, olive oil, peppermint oil, isopropyl myristate or hexylene glycol. HPE-101 significantly enhanced the excretion of [^{14}C]indomethacin, [^{14}C]nicotinic acid, [^{14}C]5-fluorouracil, [^{3}H]oestradiol and [^{3}H]triamcinolone acetonide, but not that of [^{3}H]testosterone. HPE-101 also significantly enhanced the excretion of [^{14}C]indomethacin applied to intact skin of rabbit, guinea-pig and rat, and to tape-stripped skin of guinea-pig, but did not enhance the excretion of [^{14}C]indomethacin applied to tape-stripped skin of rat or rabbit.

In previous studies (Yano et al 1992), we demonstrated that 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101) markedly enhanced the percutaneous absorption of indomethacin in hairless mice. When a substance is percutaneously absorbed, the amount and the rate of percutaneous absorption may be determined by the vehicle, the penetrant and the animal species used (Ziegenmeyer 1982). In this study we investigated the influence of these factors on the skin penetration-enhancing activity of HPE-101.

Materials and Methods

Penetrant, vehicle and penetration enhancer

[^{14}C]Indomethacin (sp. act. 1.48 GBq mmol^{-1}), [^{3}H]testosterone (sp. act. 3.66 GBq mmol^{-1}), [^{3}H]oestradiol (sp. act. 1.72 GBq mmol^{-1}) and [^{3}H]triamcinolone acetonide (sp. act. 1.61 GBq mmol^{-1}) were obtained from New England Nuclear (MA, USA). [^{14}C]Nicotinic acid (sp. act. 1.95 GBq mmol^{-1}) and [^{14}C]5-fluorouracil (sp. act. 2.07 GBq mmol^{-1}) were from Amersham Japan (Tokyo, Japan). All the vehicles and reagents used were of analytical grade and were obtained from Nakarai Tesque Co. (Kyoto, Japan), and Wako Pure Chemicals Co. (Osaka, Japan). HPE-101 and 1-dodecylazacycloheptan-2-one (laurocapram) used as penetration enhancers were synthesized in Hisamitsu Research Laboratories.

Animals

Intact animal skin. Female hairless mice of HRS/J(hr) (8–9 weeks old, 21–27 g; Kyudo, Kumamoto, Japan), male Wistar rats (Kyudo) 162–167 g, male guinea-pigs (Kyudo) 350–420 g, and male Japan White rabbits (Kyudo) 2·28–2·82 kg were housed in a room kept at a temperature of 24–26°C and relative humidity of 40–70% and had free access to commercial solid diets (CE-2, CG-3 and CR-3; Clea Japan, Tokyo, Japan) and tap water. The mice and rats were maintained on CE-2, the guinea-pigs on CG-3, and the rabbits on CR-3.

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Tape-stripped animal skin. A commercial adhesive tape (Nichiban Co., Tokyo, Japan) was applied to and removed from the dorsal skin of the intact animals. This procedure was repeated fifteen times to strip off the stratum corneum (tape-stripped or stripped animal skin).

Preparation of patches

The test patches for hairless mice were prepared as described previously (Yano et al 1992). The radioactive test drugs diluted with the respective non-radioactive test drugs were dissolved in solvents to give penetrant solutions. The penetration enhancers were dissolved in the penetrant solutions to give enhanced solutions. Fifty microlitres of different enhanced solutions were applied to a 1 × 1 cm pad fixed onto a 3 × 3 cm adhesive tape (Torii & Co., Tokyo, Japan) to prepare the test (enhanced) patch. When steroids were used as penetrants, 100 μL of the enhanced solutions were applied to 2 cm^2 pads (Torii & Co., Tokyo, Japan). The penetrant solutions without enhancer were used as the control solutions, and the control patches were prepared by applying the control solutions in the same way as the enhanced patches. The test drugs, concentration of drugs, vehicles and vehicle volume/animal are shown in Table 1.

The test and the control patches for the other animal species were prepared by incorporating the test and the penetrant solutions into patches of specified sizes. The animal species, dose applied, application area and vehicle volume/animal are shown in Table 2.

Excretion of radioactivity in urine

The test and control patches were applied to the dorsal skin of the animals to give treated and control groups, respectively. The animals were transferred to metabolic cages, and urine collected for 24 h after application of the patches. The collected urine samples were diluted with water to 30 or 300 mL, then 10 mL of liquid scintillator ACS II (Amersham, Japan) was added to 1 mL of the diluted urine samples, and radioactivity was measured in a liquid scintillation spectrometer (Tri-Carb 4530; Packard Int. Co, IL, USA) to determine the amount of percutaneously absorbed penetrant, which was expressed as percent of the dose applied.

Table 1. Test drug, concentration of drug, vehicle and vehicle volume/animal.

Test drug	Concn (mg mL^{-1})	Vehicle	Vehicle volume/ animal (μL)
[^{14}C]Indomethacin	0·5	Propylene glycol:ethanol (9:1)	50
[^{14}C]Nicotinic acid	0·5	Propylene glycol:ethanol (9:1)	50
[^{14}C]5-Fluorouracil	1·0	Ethanol:water (9:1)	50
[^{3}H]Testosterone	0·5	Propylene glycol:ethanol (9:1)	100
[^{3}H]Oestradiol	0·5	Propylene glycol:ethanol (9:1)	100
[^{3}H]Triamcinolone acetonide	0·5	Propylene glycol:ethanol (9:1)	100

Table 2. Animal species, dose applied, application area and vehicle volume/animal.

Animal species	Dose applied (mg mL^{-1})	Application area (cm^2)	Vehicle volume/ animal (μL)
Rabbit	10·5	42·0	2100
Guinea-pig	1·5	6·0	300
Rat	1·0	4·0	200
Hairless mouse	0·25	1·0 or 2·0	50 or 100

Data were expressed as the means ± s.e. of at least three determinations and analysed with Student's *t*-test.

Influence of vehicle

To make different penetrant solutions [^{14}C]indomethacin was dissolved in both hydrophilic and lipophilic solvents, each containing 10% ethanol (v/v). HPE-101 was dissolved in the penetrant solutions to give 3% (w/w) enhanced solutions. The enhanced and the control patches were prepared with the enhanced and the penetrant solutions, respectively, and they were applied to the dorsal skin of hairless mouse. The radioactivity excreted in 24-h urine samples was measured as described above.

Influence of test drug

The test drugs shown in Table 1 were dissolved in the propylene glycol:ethanol (9:1) to give penetrant solutions. HPE-101 and laurocapram were dissolved in the patches as described above, with the test and the penetrant solutions, respectively, and applied to the intact and the tape-stripped dorsal skin of the hairless mice. The radioactivity excreted in 24-h urine samples was measured as described above.

Influence of animal species

[^{14}C]Indomethacin was dissolved in a propylene glycol:ethanol (9:1) mixture to give a penetrant solution. HPE-101 and laurocapram were each dissolved in the penetrant solution to

Table 3. Urinary excretion of radioactivity following application of [^{14}C]indomethacin to skin of hairless mouse.

	Excretion in 24 h (% dose)	
Vehicle	Control	With HPE-101
Dipropylene glycol	0·9 ± 0·2	19·0 ± 6·1
Triethylene glycol	0·4 ± 0·1	26·8 ± 3·6**
Hexylene glycol	8·1 ± 0·7	12·5 ± 4·1
Diethylene glycol	2·1 ± 0·2	31·9 ± 1·9**
1,3-Butylene glycol	1·9 ± 0·6	18·0 ± 3·2*
Trimethylene glycol	1·6 ± 0·5	34·0 ± 4·5**
Glycerin	1·6 ± 0·4	13·1 ± 1·9**
Water	9·2 ± 0·3	28·4 ± 3·0**
Oleyl alcohol	9·8 ± 1·4	8·4 ± 3·0
Isopropyl alcohol	20·6 ± 2·6	19·8 ± 2·1
Ethanol	21·6 ± 1·8	16·8 ± 4·6
Silicone	4·3 ± 0·9	10·6 ± 0·8**
Peppermint oil	30·9 ± 3·2	33·5 ± 3·6
Olive oil	2·3 ± 0·1	1·8 ± 0·3
Isopropyl myristate	6·9 ± 0·5	5·5 ± 0·9
Triethanolamine	6·4 ± 3·8	37·6 ± 7·5**

All values are means ± s.e. (n = 4–5).
*$P < 0.05$, **$P < 0.01$ compared with corresponding control.

Table 4. Urinary excretion of radioactivity following application of labelled drugs to intact and stripped skin of hairless mouse.

	Excretion in 24 h (% dose)			
			Enhancer	
Drug applied	Stripped skin	Control	HPE-101	Laurocapram
[^{14}C]Indomethacin	32·5 ± 4·0**	4·0 ± 0·5	30·2 ± 3·1**	32·7 ± 2·2**
[^{14}C]Nicotinic acid	23·9 ± 2·1**	2·1 ± 1·0	15·5 ± 2·3**	20·2 ± 3·3**
[^{14}C]5-Fluorouracil	82·4 ± 3·0**	22·2 ± 5·0	65·3 ± 6·2**	85·6 ± 1·1**
[^{3}H]Testosterone	39·1 ± 2·0*	17·1 ± 5·3	34·7 ± 4·5	35·2 ± 1·0
[^{3}H]Oestradiol	22·8 ± 2·9	6·6 ± 1·2	10·5 ± 0·9*	9·3 ± 0·8
[^{3}H]Triamcinolone acetonide	8·1 ± 1·0**	1·1 ± 0·1	6·5 ± 0·4**	4·7 ± 0·2**

All values are means ± s.e. (n = 3–4).
*$P < 0.05$, **$P < 0.01$ compared with corresponding control.

Table 5. Urinary excretion of radioactivity following application of [^{14}C]indomethacin to skin of rabbit, guinea-pig and rat.

	Excretion in 24 h (% dose)					
	Control		With HPE-101		With laurocapram	
Animal	Intact	Stripped	Intact	Stripped	Intact	Stripped
Rabbit	11·6±3·2	53·5±5·1	35·8±0·6*	54·7±2·2	60·0±3·7*	55·0±5·5
Guinea-pig	0·8±0·2	24·1±0·9	14·7±3·2*	36·3±1·0**	14·3±1·6**	34·0±2·9*
Rat	1·5±0·2	22·7±2·0	21·9±2·4**	25·9±1·8	17·9±1·5**	28·2±1·2

All values are means±s.e. (n=3–4).
*$P<0·05$, **$P<0·01$ compared with corresponding control.

give 3% (w/w) test solutions. The test and the control patches were prepared in the same way as described above, with the test and the penetrant solutions, respectively. The dorsal skins of rats, guinea-pigs and rabbits were depilated by an electric clipper and electric razor the day before the experiment. The enhanced and the control patches were applied to both intact and tape-stripped dorsal skins of the animals. The radioactivity excreted in 24-h urine samples was measured as described above.

Results

The results are summarized in Tables 3–5.

Discussion

A vehicle containing a chemical enhancer should be chosen in such a way that it results in high penetration enhancement of the drug and low irritation to the skin. HPE-101 had a marked enhancing activity when used with glycols, including propylene glycol. These glycols are widely used as humectants in pharmaceutical and cosmetic formulations. Propylene glycol, in particular, has often been used with chemical enhancers (Cooper 1984). Laurocapram is a well-known chemical enhancer whose effectiveness was compared in this study with the effectiveness of HPE-101. Some reports have shown that the concomitant use of laurocapram with propylene glycol produced a synergistic activity in enhancing the penetration of the drugs through the skin (Wotton et al 1985; Touitou & Abed 1985), but the use of laurocapram with high concentrations of propylene glycol irritates the skin (Vaidyanathan et al 1987). Several humectants are also reported to cause irritation to the skin. 1,3-Butylene glycol and glycerin are reported to be less irritating than propylene glycol (Motoyoshi et al 1984). HPE-101 produced the penetration-enhancing activity in these vehicles, but these enhancements were less than those obtained when using a propylene glycol vehicle.

Okamoto et al (1990) suggested that the vehicles change the penetration-enhancing activity of penetration enhancers. In both water (hydrophilic) and silicone (lipophilic), HPE-101 produced a significant penetration-enhancing activity. Adachi et al (1988) found that the penetration-enhancing activity of laurocapram was directly proportional to the amount of laurocapram present in the skin. Sloan et al (1986) reported the effect of vehicles on the penetration of drugs (or enhancer compounds) and concluded that poor solubility of the compound in the vehicle produced high partitioning of the compound into the skin. HPE-101 is insoluble in both water and silicone and is likely to partition easily into the stratum corneum.

The following vehicles are reported to act as enhancers: ethanol (Ghanem et al 1987), isopropyl myristate (Sato et al 1988), oleyl alcohol (Cooper 1984), and peppermint oil including 1-menthol (Hori et al 1987). The penetration of indomethacin from each of the above vehicles was relatively high compared with penetration from the other vehicles used in this study without chemical enhancers. In those vehicles which act as enhancers by themselves, HPE-101 did not show enhancing activity.

Aungst et al (1990) reported that triethylamine promoted the percutaneous absorption of indomethacin. It is suggested that triethanolamine, a basic vehicle used in this study, could similarly promote the percutaneous absorption of indomethacin, making an ion pair between both compounds. HPE-101 had penetration-enhancing activity in triethanolamine vehicle in contrast to the other vehicles. This difference was probably due to the fact that the other vehicles alter the physicochemical properties of the stratum corneum whereas triethanolamine, which is a basic vehicle, interacted with indomethacin.

Barry & Bennett (1987) classified chemical enhancers on the basis of the class of penetrants they enhance. Laurocapram and propylene glycol have been shown to enhance the percutaneous penetration of a number of hydrophobic and hydrophilic compounds. Cooper (1984) reported that oleic acid and propylene glycol promote the percutaneous absorption of non-polar drugs. Decylmethyl sulphoxide, in general, enhances polar drug permeation more dramatically than the penetration of non-polar drugs (Cooper 1982; Barry & Bennett 1987). In this study HPE-101 was used with various penetrants in order to observe their penetration-enhancing effects by determination of the spectra of those penetrants. HPE-101 enhanced the penetration of both a hydrophilic drug (5-fluorouracil, log P (n-octanol/water) = −0·95 (Leo et al 1971)) and a lipophilic drug (indomethacin, log P (n-octanol/water) = 4·42 (Inagi et al 1981)). No marked difference was noted between the penetration enhancement spectra of HPE-101 and laurocapram. These results suggest that HPE-101 may work like laurocapram in terms of skin penetration enhancement.

Some reports have dealt with species differences in the

penetration-enhancing activity of enhancers (Catz & Friend 1990; Hirvonen et al 1991). HPE-101 and laurocapram produced different enhancing activities on different animal species, implying that the difference in enhancing activity would come from the differences in dermal thickness and structure among the animal species (Sugibayashi et al 1985; Elling 1986). HPE-101 and laurocapram applied to the tape-stripped skins produced no significant penetration-enhancing activity as compared with application to the intact animal skins (Table 5). This suggests that the site of action of these penetration enhancers is principally the stratum corneum (Kao et al 1985).

Laurocapram and HPE-101 have penetration-enhancing activity in tape-stripped guinea-pig, but not in stripped skin from rabbit or rat. Therefore, it is likely that fifteen tape strips of guinea-pig skin is insufficient to remove completely the stratum corneum, thus leaving some remnants of the barrier with which laurocapram and HPE-101 can interact.

Hori et al (1991) pointed out that percutaneous absorption involves drug partitioning from the vehicle into the stratum corneum, drug diffusion through the stratum corneum, drug partitioning from the stratum corneum to the aqueous viable tissue, and drug diffusion through the viable tissue to the dermal microcirculation. Furthermore, Beastall et al (1988) reported that penetration enhancers may act by altering the diffusion characteristics of the skin or by modifying the partitioning behaviour of the drug at the stratum corneum-viable epidermal interface. Laurocapram and HPE-101 are both lipophilic compounds having log P values (n-octanol/water) of 6·2 (Brain et al 1991) and 5·0 (Nakao et al 1989), respectively. Guy & Hadgraft (1988) have reported the nature of the stratum corneum as a lipid-rich barrier. The mechanism of the penetration-enhancing action of laurocapram, therefore, appears to be due to its interaction with the lipid matrix of the stratum corneum (Beastall et al 1988). In this study HPE-101 showed almost the same penetration-enhancing pattern as did laurocapram. It is, therefore, suggested that the mechanism of penetration-enhancing action of HPE-101 is the same as laurocapram.

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