ELSEVIER

International Journal of Pharmaceutics 126 (1995) 35–40

international journal of pharmaceutics

Preparation of *N*-adamantyl *n*-alkanamides and evaluation of their transdermal penetration in the rabbit

Suk Kyu Han[a,*], Young Hun Park[a], Chong Kook Kim[b]

[a]*College of Pharmacy, Pusan National University, Changjeun-Dong, Kumjung-Gu, Pusan, 609-735, Korea*
[b]*College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Ku, Seoul, 151-742, Korea*

Received 29 September 1994; revision received 14 January 1995; accepted 24 February 1995

Abstract

Novel eight *N*-adamantyl *n*-alkanamides were synthesized by amide condensation reaction between amantadine and *n*-alkanoic acid(C_7–C_{13}). Their enhancing activity on the penetration of salicylic acid through rabbit skin from petrolatum ointment was evaluated in in vivo experiment. The results of the experiment showed that the compounds have a strong transdermal penetration-enhancing activity, and their activity was parabolically dependent on the length of the alkyl chain. The mechanism of the transdermal penetration-enhancing activity of the compounds was ascribed to the reduction of the resistance to drug flux of the stratum corneum lipid layers due to the loose packing of the layers when the bulky head group of the enhancers inserts into the layers.

Keywords: *N*-Adamantyl *n*-alkanamide; Transdermal penetration enhancer; Salicylic acid

1. Introduction

Up to date, dermatological preparations have been used with drugs intended principally for local therapy. In recent years, however, the transdermal route has been recognized as a noteworthy alternative for systemic drug delivery with several advantages over conventional routes. However, most drugs do not penetrate the skin at a sufficient rate for therapeutic availability, and only a very limited number of drugs have been successful for a transdermal dosage form. Drug delivery via skin is not a simple task. The absorption rate of drugs through the skin is generally much slower than through the gastrointestinal tract. In order to overcome the low bioavailability, methods to improve transdermal delivery of drugs have been the focus of pharmaceutical research (Li and Robinson, 1987; Barry, 1987).

Many experiments have shown that the intact stratum corneum is the main barrier restricting skin permeability of drugs (Bisset, 1987). One approach to improve the low skin penetrability of the drugs is the use of penetration enhancers to reduce the barrier function of skin, which interacts with stratum corneum constituents, disrupting the highly ordered structure of the layer. The

* Corresponding Author.

SSDI 0378-5173(95)04072-I

compounds used for this purpose are of diverse structures and properties (Barry, 1983), and the mechanism of transdermal penetration-enhancing activity has been extensively investigated, and thus is not completely understood and controversial.

Concerning molecular structure, it has been noted that many transdermal penetration enhancers characteristically possess both a long alkyl chain group and a cyclic bulky moiety. Some compounds such as alkylazacycloheptanone (Azon) (Stoughton, 1982), pyrollidone derivatives (Sasaki et al., 1991), cyclohexanone derivatives (Quan, 1989a, Quan, 1989b, Quan, 1991), cyclic urea (Wong et al., 1988; Wong et al., 1989), and cyclic sulfoxide derivatives (Aoyagi et al., 1991) belong to this category. They are supposed to fluidize the lipid layers of the stratum corneum and to reduce the resistance to the flux of drugs due to the loose packing when they incorporate into the layers. A striking parallelism between transdermal drug flux and the fluidity of the stratum corneum lipid has been reported (Golden et al., 1987).

In this article, novel transdermal penetration enhancers that possess tricyclodecane as a cyclic bulky group and a long alkyl chain were prepared. Their transdermal penetration-enhancing activities were investigated by measuring their effects on the in vivo skin permeation of salicylic acid through rabbit skin.

2. Materials and methods

2.1. Chemicals

Amantadine, *n*-alkanoic acids and ethylchloroformate were obtained from Sigma Chemical Co. (USA). Chloroform, triethylamine, salicylic acid and white vaseline were purchased from Junsei Gagaku Co. (Japan), and dimethylsulfoxide (DMSO) from Yakuri Gagaku Co. (Japan). Azone was a gift from Nelson-Sumisho Co. (Japan). All other chemicals were of the highest grade available.

2.2. Instruments

Melting points were measured with Electrothermal Digital Melting Point Apparatus model FA 9100. IR spectra were recorded on a Bomem MB-100 FT-IR with KBr disk method and UV spectra were measured with Varian DMS 90 UV-VIS spectrophotometer. ^{1}H-NMR spectra of compounds were measured in $CDCl_3$ on Joel-GX 400 spectrometer. The chemical shifts were recorded as units relative to tetramethylsilane as the internal standard. Mass spectra were measured with Joel, double-focus, JMSD-300 mass spectrometer.

2.3. Synthesis and purification of adamantyl *n*-alkanamides

As shown in Scheme I, *N*-adamantyl *n*-alkanamides (AD-C_n, $n = 7-14$) have been prepared by condensation of the *n*-alkanoic acids with amantadine through the mixed carboxylic-carbonic anhydride using ethylchloroformate (Perron et al., 1960). The synthesized crude substances were purified by passing through the silica gel column and by recrystallization in hot acetonitrile/acetone (50/50). The homogeneity of the compounds was checked by thin layer chromatography and by gas chromatography. The melting points and yields of the products are listed in Table 1.

2.4. Preparation of petrolatum ointments

The test ointments used for percutaneous absorption were prepared as follows. The enhancer and the drug were dissolved in DMSO, and white vaseline was separately liquefied by warming at 70°C. Both solutions were mixed and stirred with a homogenizer. The composition of the petrolatum ointment was as follows; 100 g of ointment contains 1.6 mmol of enhancer (Ad-C_{11}, Azone or, *n*-decanoic acid), 5.0 (w/w)% DMSO and 10.0 (w/w)% salicylic acid. DMSO was added for solubilization of the enhancer and the drug in the ointment. The solubilization was ascertained by microscopic observation of the ointment at 20°C.

Table 1
Strucutral formulae, general names, melting points and yields of *N*-adamantyl *n*-alkanamides

Abbreviation	R	General name	mp[a] (°C)	Yield (%)
Ad-C7	$-(CH_2)_5CH_3$	*N*-adamantyl *n*-heptanamide	73–74	75.6
Ad-C8	$-(CH_2)_6CH_3$	*N*-adamantyl *n*-octanamide	69–70	78.6
Ad-C9	$-(CH_2)_7CH_3$	*N*-adamantyl *n*-nonanamide	60–61	75.3
Ad-C10	$-(CH_2)_8CH_3$	*N*-adamantyl *n*-decanamide	73–74	82.4
Ad-C11	$-(CH_2)_9CH_3$	*N*-adamantyl *n*-undecanamide	63–64	78.4
Ad-C12	$-(CH_2)_{10}CH_3$	*N*-adamantyl *n*-dodecanamide	75–76	85.2
Ad-C13	$-(CH_2)_{11}CH_3$	*N*-adamantyl *n*-tridecanamide	71–72	82.1
Ad-C14	$-(CH_2)_{12}CH_3$	*N*-adamantyl *n*-tetradecanamide	77–79	87.6

[a]Uncorrected melting point.

2.5. Application of petrolatum ointments on the rabbit skin

Each set of ointment was applied to two pairs of New Zealand White rabbits weighing between 2.5 and 3.5 kg. Each rabbit was used only four times. A 7-day rest period ensued before reapplication of ointment. The rabbit receiving the test ointment for the first test run received the control ointment for second run and vice versa. The animals were maintained on Purina rabbit chow and water ad libitum and housed individually in an animal room maintained at approximately 20°C, and at a relative humidity of approximately 50%. On the day the experiments were performed, the hair on both sides of the spine in the dorsal area of the rabbit skin was removed carefully with scissors and electric clippers. The edge of an 8×10 (cm^2) was designated by attaching a adhesive tape to produce a rectangle. An accurately weighed 5.0-g sample of the selected ointment was uniformly spread over shaved back skin of the rabbit, and adjusted to conform to the contour of the applied area. To ensure adequate contact between the ointment and the skin, and to minimize contamination, the applied site was immediately occluded with wrap film and then wrapped with an adhesive bandage. The ointment remained in contact with the skin for the 5-h experimental period, during which time the rabbit did not receive food and water. On completion of a test, the rabbit had the application removed, and the tested area was thoroughly washed with the warm water and detergent five to ten times and dried.

2.6. Determination of drugs in plasma

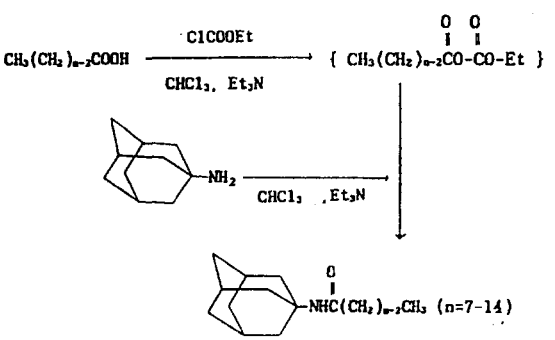
Blood samples were withdrawn and tested for salicylic acid concentration in plasma. One-half milliliter of blood was withdrawn from the marginal ear vein of the rabbit at the following times; 0.5 h prior to the application of ointment, 0.5 h after ointment application, and at hourly intervals for 6 h after ointment application. The blood was withdrawn into a syringe containing 0.1 ml of heparin (1000 IU/ml) in a centrifuge tube. This blood and heparin mixture was centrifuged for 10 min, and 0.5 ml of the separated plasma was taken into a test tube. Salicylic acid in plasma was determined by the method of Trinder (1954); 1.0 ml of the distilled water and 2.0 ml of Trinder's reagent was added to the plasma sample. After vortex-mixing and centrifuging, the absorbance of the supernatant was measured at 540 nm. The

absorbance reading obtained from the blood sample withdrawn prior to application of ointment was adjusted to the zero reading. The salicylic acid content of the sample was obtained from the calibration curve.

3. Results and discussion

The amide synthesis reaction employed in this research is a simple one, and eight novel *N*-adamantyl *n*-alkanamides were prepared with relatively high yields. The synthetic diagrams are shown in Scheme 1. The products were practically insoluble in water, slightly soluble in glycerin and propylene glycol, and moderately soluble in organic solvents such as chloroform, ethanol, etc., indicating that they are highly lipophilic compounds.

Fig. 1 shows the penetration profiles of salicylic acid through rabbit skin from petrolatum ointment in the presence of *N*-adamantyl *n*-alkanamides as transdermal penetration enhancers. Their penetration-enhancing activities were compared with those of the *n*-decanoic acid, and Azone, which are relatively well established as transdermal penetration enhancers. It was apparent that the initial rates of penetration of the drugs were greatly increased by the presence of these enhancers in the vehicle. This means that these compounds have strong transdermal penetration-enhancing activities. All *N*-adamantyl *n*-alkanamides prepared in this research showed



Scheme 1. Synthesis of *N*-adamantyl *n*-alkanamides.

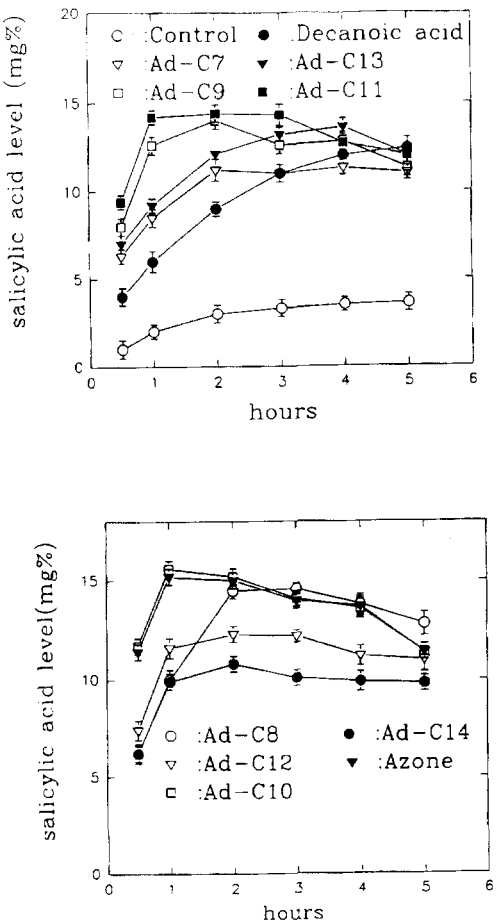


Fig. 1. Plasma concentration of salicylic acid as a function of time after transdermal administration of petrolatum ointment through rabbit skin. Each value represents the mean ± S.D. of three experiments.

stronger penetration-enhancing activities than *n*-decanoic acid. Especially, the activity of *N*-adamantyl *n*-decanamide, which was the most effective among the the enhancers prepared in this experiment, was comparable to that of Azone, which is known to be one of the most prominent penetration enhancers ever reported.

The pharmacokinetic parameters of salicylic acid after percutaneous administration under the experimental condition are shown in Table 2, which lists the maximum concentration of the drugs in plasma (C_{max}), the time for required for the maximum concentration in blood (t_{max}) and

the area under curve (AUC) of the plasma concentration-versus-time curve during initial 5-h period. These data also show that *N*-adamantyl *n*-alkanamides significantly increased the transdermal penetration rates of salicylic acid through rabbit skin from petrolatum ointment; they increased the initial rates of penetration of drugs, increased C_{max}, decreased t_{max}, and also increased AUC. In the presence of *n*-decanoic acid, a moderate transdermal penetration enhancer, the concentration of salicylic acid in plasma increased steadily, but did not reach the C_{max} within the 5-h period. However, in the presence of *N*-adamantyl *n*-alkanamides or Azone, the initial absorption rates of salicylic acid were great enough to reach C_{max} in their profiles, and their AUC values were far larger than that of *n*-decanoic acid.

Fig. 1 and the Table 2 show that the activities of *N*-adamantyl *n*-alkanamides increased as the length of the alkyl chain increased. However, the increase showed a parabolic dependence; when the length of the alkyl chain was longer than *n*-decane, the activity rather decreased. This kind of parabolic dependence has been frequently observed in the chemical structures of transdermal penetration enhancers (Scheuplein and Dugard, 1973; Irwin et al., 1990; Hori et al., 1991). This optimal chain length may be interpreted as follows; adequate length of alkyl chain is required for best incorporation of the enhancer into the stratum corneum lipid layers.

Table 2
Pharmacokinetic parameter of salicylic acid after transdermal administration through rabbit skin

Petrolatum ointment	C_{max} (mg%)	t_{max} (h)	AUC (mg%, h)
Control	ND[a]	ND	16.4 ± 2.4
Decanoic acid	ND	ND	47.2 ± 5.2
Azone	15.2 ± 2.8	1.0	70.3 ± 6.4
AD-C7	11.0 ± 1.9	4.0	52.8 ± 4.3
AD-C8	14.4 ± 2.2	3.0	64.0 ± 5.8
AD-C9	13.5 ± 3.1	2.0	63.3 ± 7.3
AD-C10	16.0 ± 2.8	1.0	76.9 ± 8.0
AD-C11	14.2 ± 2.0	2.0	70.0 ± 6.8
AD-C12	12.6 ± 4.1	2.0	60.1 ± 6.2
AD-C13	13.0 ± 3.3	4.0	60.0 ± 7.4
AD-C14	11.1 ± 3.6	2.0	42.4 ± 6.9

[a]Not detected. Each value is the mean ± S.D. of three experiments.

DMSO has been known as a transdermal penetration enhancer (Barry, 1983), and the transdermal penetration-enhancing activities observed in this experiment might be ascribed to the presence of DMSO in the ointment. However, the content ratio of DMSO employed in this experiment is relatively low 5% (w/w), and the control which contains 5% (w/w) DMSO without *N*-adamantyl *n*-alkanamide did not show as much penetration-enhancing activity as the samples. Shen et al. (1976) reported that transdermal absorption of salicylic acid from petrolatum ointment was greatly increased by nonionic surfactant in the presence of DMSO in the ointment. The role of DMSO in the transdermal penetration-enhancing activities of *N*-adamantyl *n*-alkanamides is not clear. It might be synergistic with the activities of the enhancers. The mechanism by which transdermal penetration of salicylic acid is increased by *N*-adamantyl *n*-alkanamides in the presence of DMSO is unknown.

However, when the bulky head group of the enhancers is incorporated into the stratum corneum lipid bilayers, it may induce loose packing of the layers and increase the fluidity of the layers. This may reduce the resistance to the flux of the drug and increase the transdermal penetration of the drug through the stratum corneum lipid layers.

Acknowledgements

This work was supported in part by research grant from the Research Center for New Drug Development, Seoul National University, Seoul, Korea

References

Aoyagi, T., Yamamura, M., Matsui, K. and Nogase, Y., Preparation of cyclic sulfoxide and their evaluation as transdermal penetration enhancers. *Chem. Pharm. Bull.*, 40 (1991) 1961–1963.

Barry, B.W., *Dermatological Formulations*, Marcel Dekker, New York, 1983, p. 160.

Barry, B.W., *Drug Delivery Systems*, Ellis Horwood, Chichester (England), 1987, p. 200.

Bisset, D.L., *Transdermal Delivery of Drugs*, Vol. 1, CRC Press, Boca Raton, FL, 1987, p. 29.

Golden, G.M., McKie, J.E. and Potts, R.O., Roles of stratum corneum lipid fluidity in transdermal drug flux. *J. Pharm. Sci.*, 76 (1987) 25–28.

Hori, F., Saton, S., Maibach, H.I. and Guy, R.H., Enhancement of propranolol hydrochloride and diazepam skin absorption in vitro: Effect of enhancer lipophilicity. *J. Pharm. Sci.*, 80 (1991) 32–35.

Irwin, W.J., Sanderson, F.D. and Po, A.L.W., Percutaneous absorption of ibuprofen and naproxen: Effect of amide enhancers on transport through rat skin. *Int. J. Pharm.*, 66 (1990) 243–252.

Li, V.H.K. and Robinson, J.R., *Controlled Drug Delivery*, Marcel Dekker, New York, 1987, p. 53.

Perron, Y.G., Minor, W.F., Holdrege, C.T., Goltstein, W.J., Godfrey, J.C., Crast, L.B., Babel, R.B. and Cheney, L.C., Derivatives of 6-aminopenicillanic acid. I. Partially Synthetic penicillins prepared from α- aryloxyalkanoic acids. *J. Am. Chem. Soc.*, 82 (1960) 3934–3938.

Quan, D., Higuchi, H.I., Takayama, K., Higashiyama, K. and Nagai, T., Promoting effect of 2-*n*-alkyl-cyclohexanone on the percutaneous absorption of indomethacin. *Drug Res. Delivery*, 5 (1989a) 149–157.

Quan, D., Takayama, K., Mitsuzono, T., Isowa, K. and Nagagi, T., Influence of novel percutaneous absorption enhancers, cyclohexanone and piperidone derivatives on histology of rat skin. *Int. J. Pharm.*, 68 (1991) 239–253.

Quan, D., Takayama, K. and Nagagi, T., Effect of cyclohexane derivatives on in vitro percutaneous absorption of indomethacin. *Drug Res. Delivery*, 4 (1989b) 323–330.

Sasaki, H., Kojima, M., Mori, Y., Nakamura, J. and Shibasaki, J., Enhancing effect of pyrrolidone derivatives on transdermal penetration of 5-fluorouracil, triamcinolone acetonide, indomethacin and flurbiprofen. *J. Pharm. Sci.*, 80 (1991) 533–538.

Scheuplein, R.J., and Dugard, P.H., Effects of ionic surfactants on the permeability of human epidermis: an eletrometric study. *J. Invest. Dermatol.*, 60 (1973) 263–268.

Shen, W., Danti, A.G. and Bruscato, F.N., Effect of nonionic surfactants on percutaneous absorption of salicylic acid and sodium salicylate in the presence of dimethylsulfoxide. *J. Pharm. Sci.*, 65 (1976) 1780–1783.

Stoughton, R.B., Enhanced percutaneous penetration with 1-dodecylazacycloheptane-2-one. *Arch. Dermatol.*, 118 (1982) 474–477.

Trinder, P., Rapid determination of salicylate in biological fluids. *J. Am. Pharm. Assoc.*, 57 (1954) 301–303.

Wong, O., Huntington, J., Konishi, R., Rytting, J.H. and Higuchi, T., Unsaturated cyclic ureas as non-toxic biodegradable transdermal penetration enhancers. I. Synthesis. *J. Pharm., Sci.*, 77 (1988) 967–971.

Wong, O., Tsuzuki, N., Ngheim, B., Kuenhoff, J., Iton, T., Masaki, N., Huntington, J., Konishi, R., Rytting, J.H. and Higuchi, T., Unsaturated cyclic ureas as non-toxic biodegradable transdermal penetration enhancers. II. Evaluation study. *Int. J. Pharm.*, 52 (1989) 191–202.