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Transdermal Permeation-enhancing Actitivity of *N*-adamantyl *n*-Alkanamides for Ibuprofen in the Rabbit

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Four *N*-adamantyl *n*-alkanamides were prepared by amide condensation reaction between amantadine and n-alkanoic acid. Their enhancing activity on the penetration of ibuprofen through rabbit skin from petrolatum ointment was evaluated in in-vivo experiment. The experiments showed that the compounds have a strong transdermal penetration-enhancing activity, and their activities were comparable with that of Azone. The measurements of the fluorescence polarization of DPH-labelled DPPC liposomes showed that these compounds considerablly decreased the phase transition temperature of the liposomes. The mechanism of the transdermal penetration-enhancing activity of the compounds was ascribed to the reduction of the resisitance to drug flux of the stratum corneum lipid layers due to the loose packing of the layers when the bulk head group of the enhancers inserts into the layers.

Key words : *N*-adamantyl *n*-alkanamides, Transdermal permeation, Fluorescence polarization

INTRODUCTION

In the last decade, there has been continuous increase in interest in the development of transdermal drug delivery systems which offer a noteworthy alternative for systemic drug delivery. This type of delivery system offers many advantages over conventional routes, such as bypassing the "first pass" effect and ease of self-administration. However, it also has its limitations.The disadvantages of transdermal delivery system have also been reviewed (Chien, 1987, Kydonieus,1987). One of the problems encountered with transdermal drug delivery is that 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. 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 many pharmaceutical researches (Li and Robinson, 1987; Barry, 1987).

The stratum corneum in the epidermis is recognized to be the predominant diffusional barrier for transdermal drug delivery(Bisset,1987). Anatomically, the stratum corneum is a multilaminated membrane consisting of lipids and proteins in a complex interlocking structure. Three pathways are suggested for drug penetration through skin; polar, nonpolar, and polar/nonpolar. Approaches to imporve the low skin permeability and availability of drugs are the use of penetration enhancers and mechanical devices such as iontophoresis and phonophoresis. Enhancers are compounds which reduce the barrier function of skin. They are supposed to interact with stratum corneum constituents, disrupting the highly ordered structure of the layer. The compounds used for this purpose are of diverse structures and properties (Barry, 1983), and the mechanism of transdermal penetration-enhancing activity is still controversial, although it has been extensively studied.

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 (Azone) (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 bilayers and to reduce the resistance to the flux of drugs due to the loose packing when they in-

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corporate into the layers.

Considering the distinctive features of chemical structures of transdermal permeation enhancers, we synthesized eight *N*-adamantyl *n*-alkanamides that possess tricyclodecane as a cyclic bulky group and a long alkyl chain and measured their effects on the *in-vivo* skin permeation of salicylic acid through rabbit skin (Han *et al.*, 1995). This article is the continuation of this work; we measured the effects of some of *N*-acamantyl *n*-alkanamides on the *in-vivo* skin permeation of ibuprofen through rabbit skin, and also their effects on the fluidity of liposomal membranes, employing the fluorescence polarization method.

MATERIALS AND METHODS

Chemicals

Amantadine, *n*-alkanoic acids and etyhlchloroformate were obtained from Sigma Chemical Co. (USA). Dipalmitoyl phosphatidyl choline (DPPC) and dipheyl hexatriene (DPH) were also supplied from Sigma Chemical Co. Chloroform, triethylamine and white vaseline were purchased from Junsei Gagaku Co. (Japan), and dimethyl sulfoxide (DMSO) from Yakuri Gagaku Co. (Japan). Azone was a gift from Nelson-Sumisho Co. (Japan). All other chemicals were of the highest grade available.

Instruments

Melting points were measured with Electrothermal Digital Melting Point Apparatus model FA9100. 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. Fluorescence was measured with Perkin-Elmer LS-5 Fluorescence Spectrophotometer.

Preparation of *N*-adamantyl *n*-alkanamides

N-adamantyl *n*-alkanamides (AD-C_n, n=8,10,12,14) were prepared by condensation of the *n*-alkanoic acids with amantadine through the mixed carboxylic-carbonic anhydride method using ethylchloroformate as described in the reference (Perron *et al.*, 1960). The synthesized crude substances were purified by passing through silica gel column and by recrystallization in hot acetonitrile/acetone (50/50). The homogeneity of the compounds was checked by thin layer chromatohrphy and by gas chromatography. The chemical structures of the prepared compounds were identified by the measurements of the melting points and by analysis of IR, NMR and mass spectra.

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_n, Azone or, *n*-decanoic acid), 5.0% (w/w) DMSO and 10.0% (w/w) ibuprofen. 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.

Application of petrolatum ointments on the rabbit skin

The 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 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 maintainied 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 4-hr 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 throughly washed with the warm water and detergent five to ten times and dried.

Determination of ibuprofen in plasma

Blood samples were withdrawn and tested for ibuprofen 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 4 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.

To 0.5 ml of the plasma, 1.5 ml of 1N HCl and 5.0 ml of n-hexane; isoamyl alcohol (95 : 5) mixture were added, and the mixture was vortexed and centrifuged. The separated organic-solvent layer was extracted with 3.0 ml of 2N NaOH, and the absorbance at 262 nm of the aqueous layer was read. The ibuprofen content of the sample was obtained from the calibration curve. The previous experiments showed that the alkaline aqueous solution of ibuprofen follows the Beer's law at this wavelength.

Measurement of fluidity of liposomal membranes

An appropriate aliquot of the *N*-adamantyl *n*-alkanamides was added to the DPPC and the lipid mixture was dissloved in chloroform. The chloroform solution was reduced to dryness by rotary evaproation, forming a film on the inside of a round-bottom flask. The small unilamellar liposome was formed by suspending the film in 10 mM Tris buffer (pH 7.4) and sonicated for 30 min above the phase transition temperature of the DPPC. The incorporation of DPH into the liposomal bilayers was carried out by adding an aliquot of 1mM stock solution of DPH (10^{-6} mM) in DMSO to the liposome. The mixture was vigorously vortexed. The sample was excited by vertically polarized light and intensities of emission are measured through polarizers, oriented paralle to the plane of excitation (IVV) and perpendicular to the plane of excitation (IVH). From these measurements, the fluorescence polarization value (P) was calculated, employing the following equation (Jones and Cossins, 1990),

$$P = \frac{IVV - IVH \times G}{IVV + IVH \times G}$$

where the correction factor, G was IHH/IVH. This value was employed as a parameter of fluidity of the liposomal membrane.

RESULTS AND DISCUSSION

Employing the amide synthesis reaction, *N*-adamantyl *n*-alkanamides were prepared with relatively high yields as described in detail n the previous report (Han *et al.*, 1995). Their melting points and yields are listed in the reference. Their chemical structures and abbreviations were shown in Table I.

Table I. Structural formulations and general names of N-adamantyl n-alkanamides

Abbreviation	R	General name
AD-C8	$-(CH_2)_6CH_3$	N-adamantyl n-octanamide
AD-C10	$-(CH_2)_8CH_3$	N-adamantyl n-decanamide
AD-C12	$-(CH_2)_{10}CH_3$	N-adamantyl n-dodecanamide
AD-C14	$-(CH_2)_{12}CH_3$	N-adamantyl n-tetradecanmide

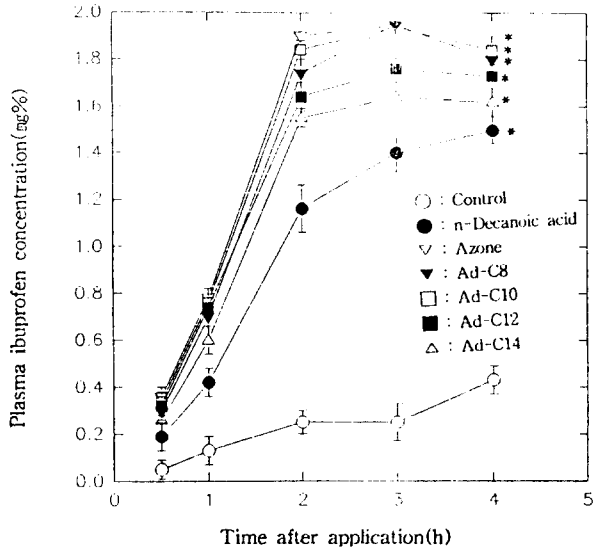

Fig. 1. Plasma concentration of ibuprofen as a functon of time after percutaneous application of petrolatum ointments through rabbit skin. Each value represents the means±S.D. of three experiments. *Singnificant difference from the control ($P<0.01$)

Fig. 1 shows the penetration profiles of ibuprofen through rabbit skin from petrolatum ointment in the presence of *N*-adamantyl *n*-alkanamides as transderaml 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 penetraton of the drugs were greatly increased by the presence of these enhancers in the vehicle ($P<0.01$). All N-adamantyl n-alkanamides prepared in this research showed stronger penetration-enhancing activities than *n*-decanoic acid. Especially, the activities of *N*-adamantyl *n*-octanamide and *n*-decanamide were comparable to that of Azone, which is known to be one of the most prominent penetration enhancers ever reported.

The pharmacokinetic parameters of ibuprofen after percutaneous administraton under the experimental condition are shown in Table II, which lists the maximum concentration in blood (C_{max}), the time required for the maximum concentration(t_{max}) and the area under curve (AUC) of the plasma concentration-versus-time curve during initial 4-h period. These data show that *N*-adamantyl *n*-alkanamides significnatly in-

Table II. Pharmacokinetic parameters of ibuprofen after transdermal administration through rabbit skin

Petrolatum ointment	C_{max} (mg%)	t_{max} (h)	AUC (mg%, h)
Contorl	ND[a]	ND	9.5±2.2
n-Decanoic acid	ND	ND	42.9±2.1
Azone	19.5±1.5	3.0	63.6±1.7
AD-C8	19.3±1.5	3.0	60.5±1.8
AD-C10	19.5±1.8	3.0	62.4±1.9
AD-C12	17.3±1.6	3.0	57.1±1.8
AD-C14	16.6±1.1	3.0	54.2±1.8

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

creased the transderaml penetration rates of ibuprofen 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 ($P<0.01$). In the presence of *n*-decanoic acid, a moderate transdermal penetration enhancer, the concertration of ibuprofen in plasma increased steadily, but did not reach the Cmax within the 4-h period. However, in the presence of *N*-adamantyl *n*-alkanamides or Azone, the initial absorption rates of ibuprofen were great enough to reach Cmax in their profiles, and their AUC values were larger than those of n-decanoic acid and the control ($P<0.99$).

The transdermal penetration experiments were terminated at 4-hr period, because the 4-hr period was long enough to observe the permeation rate-increasing activities of the compounds, and to minimize the pain and discomfort given to the animal, keeping it survived.

Fig. 1 and the Table II show that the activities of *N*-adamantyl *n*-alkanamides requires a moderate length of the alkyl chain. However, as the length was longer than n-decane, the activity rather decreased. AD-C_8 and AD-C_{10} showed the activities comparable to that of Azone, and significant differences among C_{max}, t_{max} and AUC of these compounds were not observed although AUC of AD-C_{10} is slightly smaller than that of AD-C_8. However, the AUC values of AD-C_{12} and AD-C_{14} were significantly smaller than that of AD-C_{10} ($P<0.11$). 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 of alkyl chain might be required for best incorporation of the enhancer into the stratum corneum lipid layers.

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, and this activity was ascribed to the activity of the nonionic surfactant. The role of DMSO in the transdermal penetration-enhancing activities of *N*-adamantyl *n*-alkanamides is not clear, and it might be synergistic with the activities of the enhancers.

The results of the measurements of the fluorescence polarization value of the DPH labelled into the liposomal membrane made of the lipid mixture DPPC and the enhancer were shown in Fig.2. The results showed that the values decreased gradually as the temperature increased and decreased abruptly near the phase transition temperature T_c of the liposomal membrane from crystalline to liquid crystalline state as usual cases. The midpoint of the abrupt changes which were shown as sigmoidal curves was taken as Tc. It was 40.5°C for DPPC liposome, which agreed fairly well with reported value (Houslay, 1982). The addition of the enhancers to DPPC slightly decreased the values at temperature below T_c, and increased the value above T_c. However, their differences were not significant. However, it was clearly shown that the addition of the enhancers to DPPC significantly lowered the T_c ($P<0.01$). The presence of Azone decreased Tc to 36.8°C. Decanoic acid, AD-C_8, AD-C_{10} and AD-C_{12} also significantly decreased the T_c. However, differences between their effects on this value were not great enough to be detected by this method. These results suggest that the enhancers tested in this experiment perturbed the lipid bilayers effectively, when they incorporate into the lipid bilayers of the liposomes, and their transdermal permeation-enhancing activity might be related to this lipid bilayer-purturbing activity. The lipid bilayer perturbation might induce the reduction of the barrier function of the skin.

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