Percutaneous Absorption of Indomethacin from Mixtures of Fatty Alcohol and Propylene Glycol (FAPG Bases) through Rat Skin: Effects of Oleic Acid Added to FAPG Base

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The effects of oleic acid (OA) added to mixtures of fatty alcohol and propylene glycol (FAPG bases) on the percutaneous absorption of indomethacin (ID) were investigated by using the abdominal skin of rats *in vivo*. The percutaneous absorption of propylene glycol (PG) from FAPG base was simultaneously examined.

The percutaneous absorption of ID from FAPG bases in the absence of OA was poor as compared with that from FAPG bases containing OA. It was observed that when OA was added to the vehicles in the range of 5 to 30%, the percutaneous absorption of ID from the vehicles was increased. In particular, the maximal enhancement of percutaneous absorption of ID was achieved at 5% OA. However, the enhancing effect of percutaneous absorption of ID diminished when the OA content in the vehicle exceeded 50%.

PG was readily absorbed through the rat skin from FAPG bases and its percutaneous absorption profiles were similar to those of ID. It can be presumed that PG and ID penetrate together through the skin. In addition, it was confirmed that the percutaneous absorption of ID and PG from FAPG bases was not affected by the viscosity of the vehicle.

If FAPG base is to be used as a vehicle for the purpose of percutaneous absorption of ID, OA is considered to be a useful additive.

Keywords FAPG base; oleic acid; enhancer; indomethacin; rat; percutaneous absorption; blood concentration; propylene glycol

Many attempts have been made to investigate the absorption of drugs through the skin from various dermal dosage forms such as ointment and cataplasms, in the expectation of a systemic effect as well as a topical effect, and further to avoid hepatic first-pass elimination. However, the skin itself presents an effective barrier to topically applied drugs. Thus, when a dermal dosage form is applied to the skin, it is difficult to obtain the same therapeutic efficacy of drugs as with the other dosage forms, such as oral and parenteral dosage forms. One method to improve the bioavailability after topical administration of drugs is to employ a penetration enhancer. Many substances such as Azone, dimethyl sulfoxide (DMSO), propylene glycol (PG), oleic acid (OA), and so on, have been suggested as enhancers of percutaneous absorption of drugs. Further, many investigations concerning the effects of enhancers *in vivo* and *in vitro* have been carried out. Among the enhancers, OA, an unsaturated fatty acid, is expected to be useful, since it shows less irritancy, toxicity, and odor as compared with DMSO, *etc.*[1]

However, it appears that little or no attention has been paid to the enhancing effect of OA added to topical vehicles such as ointments and cream bases on the percutaneous absorption of a drug. Thus, in order to investigate the effect of OA on the percutaneous absorption of a drug from a vehicle, we selected mixtures of fatty alcohol and propylene glycol (FAPG base) as the topical product in this study. FAPG base, which is a commercial topical vehicle, is a mixture of fatty alcohol (FA), PG and other excipients, and has been used as a topical vehicle for various corticosteroids. In the previous paper, we reported that indomethacin (ID) was readily absorbed through the rat skin from FAPG bases, and we suggested that FAPG base could be a useful vehicle for percutaneous drug administration.[2,3] In the present study, we investigated the influence of OA added to FAPG on the percutaneous absorption of ID. The efficiency of percutaneous absorption of ID was determined by measuring the drug concentration in rat plasma.

Experimental

Materials PG, OA and stearyl alcohol were purchased from Tokyo Kasei Co., Ltd. ID was obtained from Sigma Chemical Company. All the solvents used in this experiment were of reagent grade from Kanto Chemical Co., Ltd.

Preparation of FAPG Bases FAPG bases containing 1% ID were prepared according to the formulae in Table I. The stearyl alcohol and OA were heated at 75 °C, then ID dissolved in PG, previously heated to the same temperature, was added. The mixture was stirred until it congealed.

***In Vivo* Experiment** Male Wistar rats weighing 230 and 250 g were used. The hair of the abdominal region was carefully removed with electric hair clippers and an electric razor without breaking the skin before the experiments. The rat was fixed on its back, and 2 g of vehicle was spread on the skin (18 cm^2). Blood (0.3 ml) was withdrawn from the jugular vein into a syringe at predetermined intervals, and centrifuged at 3000 rpm for 10 min. The resulting plasma samples were individually subjected to ID and PG content measurement by high performance liquid chromatography (HPLC) and gas chromatography.

Assay of ID and PG The plasma concentrations of ID and PG were determined in the manner described in the previous paper.[2]

Measurement of Viscosity The Rheomat 30 cone-and-plate viscometer (Contraves Co., Ltd) was used to determine the viscosity of FAPG bases at 34 °C. The maximum shear rate was 42.1 s^{-1} with a 30 s sweep time. Apparent viscosity was obtained from the shear stress at the maximum shear rate.

TABLE I. Formulae of FAPG Bases

Composition[a]	Base No.[b]					
	1[c]	2	3	4	5	6
Stearyl alcohol	40	40	40	40	40	40
PG	60	55	50	30	10	—
OA	—	5	10	30	50	60

a) In grams. *b*) The content of ID was fixed at 1% in each base. *c*) No. 1 FAPG base is the control base in this paper.

Results and Discussion

Percutaneous Absorption of ID from FAPG Bases The plasma ID concentration–time curves after the topical administration of Nos. 1—6 bases are shown in Fig. 1. In addition, Fig. 2 depicts the relationship between the area under the plasma concentration–time curves (*AUC*s) of ID and the content of OA in the vehicle.

The percutaneous absorption rates of ID from Nos. 2, 3 and 4 FAPG bases containing OA after application were fast as compared with that of No. 1 base (without OA, control). Furthermore, the mean *AUC*s and the mean ID plasma concentration for 8 h after administration of Nos. 2, 3 and 4 FAPG bases were also statistically significantly higher than those of the control ($p<0.01$). The ID plasma level of about 32 μg/ml for No. 2 FAPG base at 8 h was in particular contrast with that of about 7 μg/ml for the control vehicle. The addition of OA to FAPG base thus clearly enhanced percutaneous ID absorption. However, exceptionally, the plasma level for 8 h and the absorption rate of ID from No. 5 FAPG base containing 50% OA after application were similar to those of the control vehicle. It was observed that the maximal enhancement of percutaneous absorption of ID was achieved by the addition of OA to FAPG base at the content of 5%. Further, the enhancement decreased as the content of OA was increased beyond 5%. No. 6 base, which was prepared with OA alone in the place of PG in the control base, showed similar behavior. The percutaneous absorption of ID after the application of No. 6 base was observed to be lower than that of the control base. The mean plasma levels at 6 and 8 h and the *AUC* in the case of No. 6 base after administration were statistically significantly lower than those of the control ($p<0.01$). The absolute availability of ID after the administration of Nos. 1, 2, 3, 4, 5 and 6 FAPG bases were 1.9, 11.1, 8.4, 5.7, 2.4 and 0.6%, respectively. It was confirmed from the above results that when OA was incorporated into FAPG base as an additive, it could enhance drug absorption, and there was an optimum content of OA giving maximal enhancement.

OA, a lipophilic material, has previously been employed to increase the percutaneous absorption of drugs, and there are many reports concerning the function of OA as an enhancer.[4-13] Golden *et al.* reported that OA treatment of stratum corneum decreased the lipid phase transition temperature, and consequently increased the motional freedom or fluidity of the lipid.[14] Barry proposed that the *cis* double bond of OA causes a kink in its alkyl chain, and thus OA creates gaps in the packed lipid structure of stratum corneum upon its application to the skin, so that the diffusional resistance of stratum corneum is reduced.[11] Barry further showed in the same report that PG, which is a

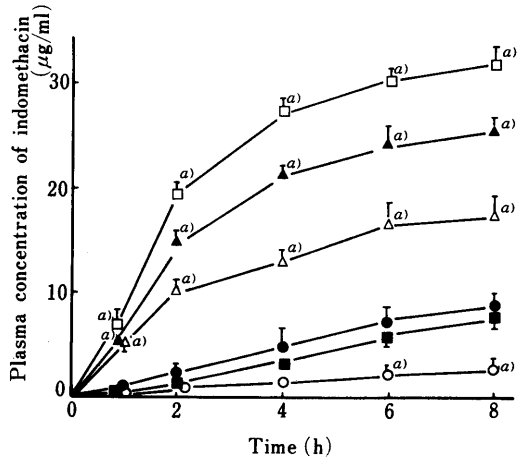

Fig. 1. Effect of the Content of OA Added to FAPG Base on Percutaneous Absorption of ID in Rats

■, No. 1 base (OA free); □, No. 2 base (5%); ▲, No. 3 base (10%); △, No. 4 base (30%); ●, No. 5 base (50%); ○, No. 6 base (60%). Each value represents the mean ± S.E. of five experiments. *a*) $p<0.01$ as compared with No. 1 base.

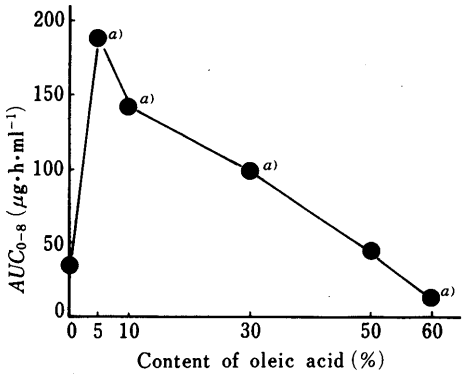

Fig. 2. Relationship between the Content of OA Added to FAPG Bases and the *AUC*s of ID after Topical Administration to Rats

a) $p<0.01$ as compared with No. 1 base.

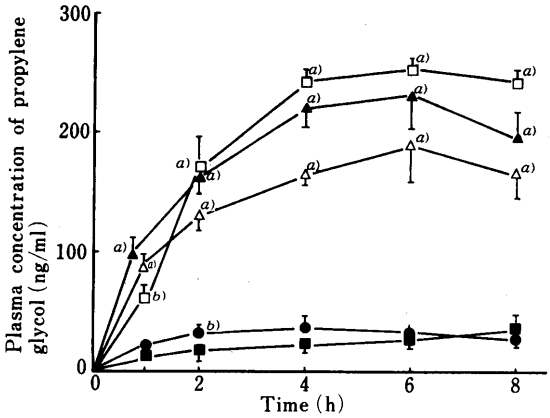

Fig. 3. Effect of the Content of OA Added to FAPG Base on Percutaneous Absorption of PG in Rats

■, No. 1 base (OA free); □, No. 2 base (5%); ▲, No. 3 base (10%); △, No. 4 base (30%); ●, No. 5 base (50%). Each value represents the mean ± S.E. of five experiments. *a*) $p<0.01$ as compared with No. 1 base. *b*) $p<0.05$ as compared with No. 1 base.

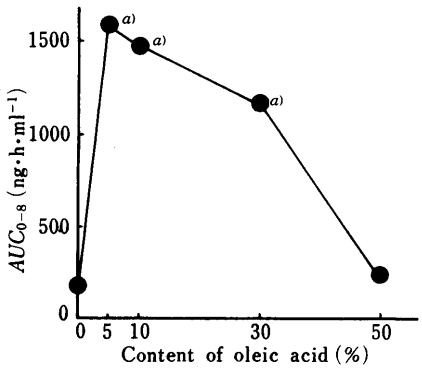

Fig. 4. Relationship between the Content of OA Added to FAPG Bases and the *AUC*s of PG after Topical Administration to Rats

a) $p<0.01$ as compared with No. 1 base.

hydrophilic material and functions as an enhancer itself, preferentially entered the keratin location in the stratum corneum at the time of administration to the skin, but did not alter the lipid fluidity. It has been elucidated that two-component systems consisting of PG and OA are effective permeation enhancers as compared with PG or OA alone.[6,9-11] This binary system is considered to disorganize the multilaminate hydrophilic–lipophilic layers located intercellularly in the stratum corneum, consequently promoting percutaneous absorption of drugs. As shown in Fig. 1, the percutaneous absorption of ID from the control base containing PG alone or No. 6 base containing OA alone was fairly low as compared with those from the vehicles containing both PG and OA, with the exception of No. 5 base. These results indicate that the percutaneous absorption of ID was a synergistic rather than a simple additive interaction between PG and OA. OA added to FAPG base was ascertained to play an important role in the percutaneous absorption of ID, although the mechanism and the reason why the maximal enhancement was observed at 5% OA are still unclear.

Percutaneous Absorption of PG from FAPG Bases Figure 3 shows the PG plasma concentration–time curves after the applications of Nos. 1—5 FAPG bases. Further, Fig. 4 depicts the relationship between the *AUC*s and the content of OA in the vehicle.

The percutaneous absorption of PG from control base was poor. On the other hand, when OA was added to the vehicles in the range of 5 to 30%, the percutaneous absorption of PG from the vehicles was increased. In particular, the percutaneous absorption of PG from No. 2 FAPG base containing 5% OA, in terms of *AUC*, was increased about 9-fold over that of the control. There was no statistically significant difference in mean plasma concentration of PG or in absorption pattern between No. 5 FAPG base containing 50% OA and the control up to 8 h after application. The enhancing effect on PG absorption was thus lost when the OA content in the vehicle exceeded 50%.

PG passes readily through excised human or animal skin from a vehicle containing PG *in vitro*.[15,16] In a previous paper, we also observed that PG was readily absorbed through the rat skin from FAPG bases and passed directly into the systemic circulation of rat.[2] It was confirmed here that PG was absorbed through rat skin from Nos. 1—5 FAPG bases, though the blood concentration of PG was fairly low as compared with that of ID. Furthermore, the mean plasma concentration–time profiles and *AUC*s of PG were similar to those of ID.

It has been reported that the percutaneous absorption of a drug from a vehicle containing PG after application involves two processes; one is drug release from the vehicle in a dissolved state in PG, and the other is subsequent drug absorption through the skin barrier together with PG.[17,18] It can be presumed from these reports and the above results that differences in the release of PG from the vehicle influence the percutaneous absorption of ID, and that PG and ID penetrate together through the skin.

Yamada *et al.* reported that OA itself was also absorbed percutaneously through the rat skin, though the amount of OA absorbed was fairly low as compared with that of PG.[9] OA is thus assumed to be absorbed percutaneously together with PG and ID from FAPG base containing OA, though this was not confirmed in our experiments.

Viscosity of Vehicles The values of apparent viscosity of Nos. 1—6 bases measured using a rheometer are shown in Table II. The viscosity of the vehicle decreased with increase of OA content and with decrease of PG content in the vehicle. The viscosity of No. 5 FAPG base containing 50% OA was about one-half that of the control in the absence of OA. It appears that the content ratio of OA/PG in the vehicle influences the viscosity of the base as well as the percutaneous absorption of ID.

Davis has found that the release of a drug from the vehicle was affected by the viscosity, and further that there was a more-or-less linear relationship between the release of the drug and the reciprocal of the viscosity.[19] This suggests that the lower the viscosity of the vehicle, the easier the release of the drug from it. On the other hand, it is apparent that the release of the drug from the vehicle influences the percutaneous absorption of the drug. That is to say, the percutaneous absorption of a drug is considered to increase with increase in the release of a drug from the vehicle. The percutaneous absorption of a drug from the vehicle may hence increase with decrease in the viscosity of the vehicle. In this study, however, it was observed that the ease of percutaneous absorption of ID through rat skin was independent of the viscosity of test vehicles. If FAPG base containing OA is eventually used in commercial products, it may be necessary to take into account the ratio of OA/PG in the vehicle to obtain the maximal therapeutic effect and to prepare a suitable vehicle in respect of viscosity.

TABLE II. Viscosity of FAPG Bases at 34 °C

	Base No.					
	1	2	3	4	5	6
Viscosity (P)	5346	4901	4039	3742	2732	2762

Each value represents the mean of three experiments.

Conclusion

It was confirmed that the addition of OA to FAPG base enhanced the percutaneous absorption of ID from FAPG base through the rat skin. In particular, a remarkable enhancement was observed at the concentrations of 5% OA and 55% PG to FAPG base. Furthermore, the percutaneous absorption of PG from FAPG bases through rat skin was observed in this study. If FAPG base is to be a vehicle for the percutaneous absorption of ID, OA would be a useful additive.

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