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The effect of pretreatment by penetration enhancers on the in vivo percutaneous absorption of piroxicam from its gel form in rabbits

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Summary

The effects of pretreatment period with 80% dimethyl sulfoxide (DMSO) and 10% oleic acid in propylene glycol (PG) on the percutaneous absorption of piroxicam from its gel form through rabbit abdominal skin were investigated. The area under the curve of plasma piroxicam concentration from 0 to 24 h (AUC_{0-24h}) for 1 h pretreatment with 10% oleic acid/PG was 8-fold higher than that of nonpretreatment, and was similar to that of 6 or 12 h pretreatment. Compared to the lag time of skin permeation of piroxicam for nonpretreatment (2 h), the lag time for the pretreatments was nearly diminished. The AUC_{0-24h} value of piroxicam for 24 h pretreatment was about 22-fold higher than that of nonpretreatment, and the absolute bioavailability for 24 h pretreatment was 62%. In contrast to 10% oleic acid/PG, 1 h pretreatment with 80% DMSO/PG had no enhancing effect on the percutaneous absorption of piroxicam from the gels. However, the AUC_{0-24h} values of piroxicam increased linearly with length of the pretreatment period from 1 to 24 h. Photomicrographs showed that the exfoliation of stratum corneum or epithelium resulted on pretreatment with 10% oleic acid for 18 h.

Introduction

Penetration enhancers are taken as additives incorporated into a formulation in order to promote the absorption of drugs from ointments or gels. Numerous approaches have been described in which DMSO and oleic acid are commonly used as effective penetration enhancers for many drugs (Hwang et al., 1983; Akhter et al., 1984; Dallas et al., 1987; Yamada et al., 1987a; Touitou et al., 1988). Incorporating a large quantity of liquid penetration enhancers such as DMSO and oleic acid into the original formulation would influence the viscosity, solubility, and other physicochemical properties of the ointments or gels. Pretreating the skin with the penetration enhancers before application of drugs would be a good method to promote the percutaneous absorption of drugs (Hosoya et al., 1987). It has been reported that the use of DMSO or oleic acid as enhancer to pretreat the skin would influence the structure of the stratum corneum, and increase the permeability of some drugs through the skin (Goodman et al., 1986; Mirejovsky et al., 1986;

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Yamada et al., 1987b; Sharata et al., 1988; Seki et al., 1989). However, only a few studies have reported on the effects of the pretreatment period on the permeability of drug through the skin (Mirejovsky et al., 1986; Yamada et al., 1987b). In the present study, DMSO/PG and oleic acid/PG were selected as penetration enhancers at their most effective concentrations of 80 and 10%, respectively (Sheuplein et al., 1971; Idson; 1975, 1985; Yamada et al., 1987a). A commercial piroxicam gel was selected as the model dosage form to investigate the relationship between the pretreatment period and the percutaneous absorption of piroxicam with the purpose of achieving an effective transdermal drug delivery system for medication of piroxicam gel or being applied to other drugs and dosage forms such as patches (Chien, 1987).

Materials and Methods

Materials

The following reagents were used: piroxicam gel (0.5%) (a gift of Heng Hsin Chemical & Pharmaceutical Co., Ltd., Taiwan, R.O.C.), piroxicam (Pfizer, U.S.A.), indomethacin (Sumitomo Chemical, Osaka, Japan), diethyl ether (E. Merck, U.S.A.), urea, oleic acid, dimethyl sulfoxide (DMSO), propylene glycol (PG), sodium phosphate dibasic, and citric acid. All other chemicals were of analytical reagent grade.

Animals

White male rabbits weighing 2.2–2.8 kg were fasted for 24 h before application of piroxicam gels and were fixed on a plate during the period of pretreatment with penetration enhancer and of application of drug.

Wet dressing method for pretreatment and topical application of piroxicam gel

The hair was removed with electric hair clippers from the skin of the abdominal region 12 h prior to application of the penetration enhancers. A piece of cotton cloth, 6×10 cm^2, was saturated and wetted with about 8 ml of 80% DMSO/PG and 10% oleic acid/PG, respectively. Then, the wetted cloth was applied to the shaved surface of the rabbit for a specific time by the occlusive dressing technique (ODT) (Naito et al., 1981). Subsequently, the wet dressing was peeled off and the applied area was gently swabbed clean 50 times with cotton to remove the residue solution without damaging the skin. Then 6 g of piroxicam gel (0.5%) was applied to the treated skin immediately by the ODT method. As a nonpretreatment, the same piroxicam gel was applied to the nontreated skin. The $AUC_{0-24\,h}$ value was calculated by using the trapezoidal rule from the plasma concentration-time curve in each experiment. All rabbit blood specimens were collected from the marginal ear vein, the plasma concentrations of piroxicam were measured at 1, 2, 4, 7, 12 and 24 h after application of the piroxicam gel and the values of C_{max} (maximum plasma concentration) were recorded for each condition, respectively. The lag time was regarded as the interval between the beginning of application of the piroxicam gel and the time at which plasma concentration of piroxicam fell below 0.1 μg/ml.

Analytic procedures

The method for the analysis of piroxicam has been described previously (Tsai et al., 1985a) with some modifications.

A 0.5 ml aliquot of plasma was pipetted into a 15 ml glass-stoppered centrifuge tube, along with 1 ml of phosphate buffer (pH 4). The mixture was shaken for 10 s and extracted with 5 ml of ethyl ether by mechanical shaking for 20 min. After centrifugation for 3 min at 3000 rpm, 4 ml of the ether phase was transferred to another tube and evaporated to dryness on a water bath at 50 °C. The residue was redissolved in 0.5 ml of mobile phase, 10 μl of indomethacin solution at a concentration of 1 mg/ml was added and then mixed for 15 s by a vortex mixer; 50 μl of this solution was then injected into the column for HPLC through a stop-flow injection port.

Histological examination of the stratum corneum

Histological changes in the stratum corneum were examined by applying 80% DMSO/PG or 10% oleic acid/PG to denuded abdominal skin, which was excised after various pretreatment peri-

ods later, fixed in formalin by the conventional procedure, stained with hematoxylin-eosin, and examined under a microscope. Untreated skin served as a control.

Bioavailability of piroxicam

An aliquot of pH 9 bicarbonate buffers containing 20 or 6 mg of piroxicam was administered intravenously to rabbits. At 0.167, 0.333, 0.667, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 h afterward, arterial blood samples were taken to determine the plasma concentration of piroxicam. The area under the plasma concentration-time curve of piroxicam (AUC^{iv}_{0-12h}) was calculated by using the trapezoidal rule, and found to be 155.69 ± 11.24 (S.E.) and 48.35 ± 3.6 (S.E.) μg h ml^{-1} for 20 and 6 mg of piroxicam, respectively. The AUC values linearly increased with increasing dose. This indicates that piroxicam may follow dose-independent pharmacokinetics. The bioavailability through the percutaneous absorption of piroxicam was determined by the following equation.

$$F_{0-24h} = \frac{[AUC]^{pr}_{0-24h}/D^{pr}}{[AUC]^{iv}_{0-\infty}/D^{iv}}$$

where F_{0-24h} is the bioavailability of piroxicam gel for percutaneous absorption of piroxicam from 0 to 24 h; $[AUC]^{pr}_{0-24h}$ the area under the curve of piroxicam gel for percutaneous absorption of piroxicam from 0 to 24 h; $[AUC]^{iv}_{0-\infty}$ the area under the curve of piroxicam for iv administration of 20 mg of piroxicam, calculated from the following equation which was obtained from a previous report (Tsai et al., 1985b): from $C = 26.03e^{-0.555t} + 14.39e^{-0.0876t}$, we obtain

$$[AUC]^{iv}_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta} = 211.2\ (\mu g\ h\ ml^{-1})$$

D^{pr} the dosage of piroxicam in 6 g of piroxicam gel (0.5%); and D^{iv} the dosage of piroxicam for iv administration, this value is 20 mg of piroxicam obtained from a previous report (Tsai et al., 1985b).

Results and Discussion

Using 80% DMSO/PG as penetration enhancer, we pretreated the skin for 1, 6, 12, and 24 h before topical application of the piroxicam gel. Then, the plasma concentration-time curves of absorbed piroxicam from 0 to 24 h for each condition and nonpretreatment were measured as shown in Fig. 1, and the AUC_{0-24h}, C_{max}, and F_{0-24h} values of absorbed piroxicam were determined, as listed in Table 1. From the results, a good relationship between the AUC_{0-24h} or C_{max} value of absorbed piroxicam and the pretreatment period was found as shown in Fig. 2. It is shown that the AUC_{0-24h} for 1 h pretreatment was not significantly different from that of nonpretreatment (t-test, $p > 0.05$). On increasing the pretreatment period from 1 to 24 h, the AUC_{0-24h} value increased linearly ($r = 0.998$). The photomicro-

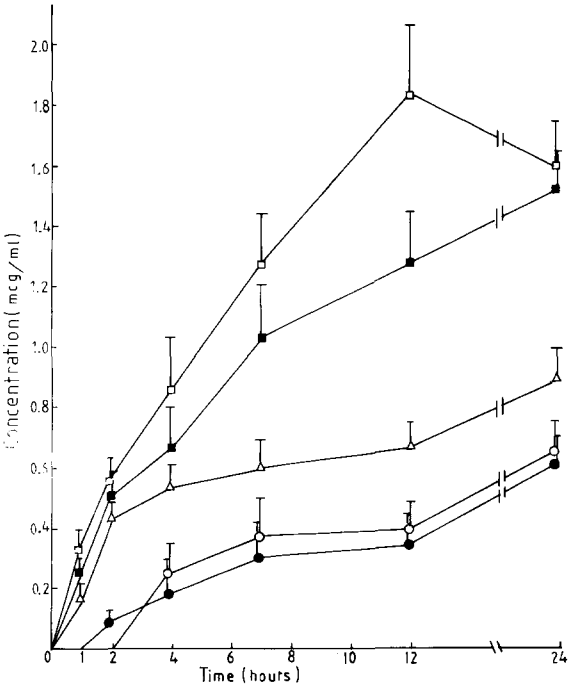

Fig. 1. Effect of pretreatment period on the plasma concentration-time curve of absorbed piroxicam by using 80% DMSO/PG as the penetration enhancer for pretreatment. (○———○) Nonpretreatment; (●———●) 1 h; (△———△) 6 h; (■———■) 12 h; (□———□) 24 h. Each point represents the mean of five rabbits with the standard error.

TABLE 1

Comparison of AUC_{0-24h}, C_{max} and F_{0-24h} values in each condition

Drug	Penetration enhancer	Pretreatment period (h)	AUC_{0-24h} ($\mu g\ h\ ml^{-1}$)	C_{max} ($\mu g/ml$)	F_{0-24h}
Piroxicam gel	none	0	9.04 ± 2.45	0.64 ± 0.08	0.029
	80% DMSO/PG	1	8.25 ± 1.16	0.62 ± 0.10	0.026
		6	15.72 ± 1.60	0.90 ± 0.12	0.050
		12	26.27 ± 4.49	1.284 ± 0.30	0.083
		24	39.16 ± 6.60	1.82 ± 0.54	0.124
	10% oleic acid/PG	0.5	45.40 ± 4.65	2.28 ± 0.27	0.143
		1	76.90 ± 13.07	4.00 ± 0.65	0.243
		6	80.97 ± 12.04	4.48 ± 0.58	0.256
		12	70.80 ± 10.38	3.36 ± 0.75	0.223
		18	113.20 ± 17.89	5.88 ± 0.88	0.357
		24	195.30 ± 29.70	11.12 ± 1.95	0.616

Data are means ± S.E. ($n = 5$).

graphs shown in Fig. 5A and B indicate that the skin pretreated with 80% DMSO/PG even for 24 h created only a slight change in the stratum corneum compared with that of nonpretreatment. Piroxicam is a lipophilic molecule with a partition coefficient (octanol/pH 7.4 buffer) of the order of 1.8 (Lombardino et al., 1973; Wiseman et al., 1982). It has been reported that the ultrastructural changes in the intercellular lamellar lipids are not

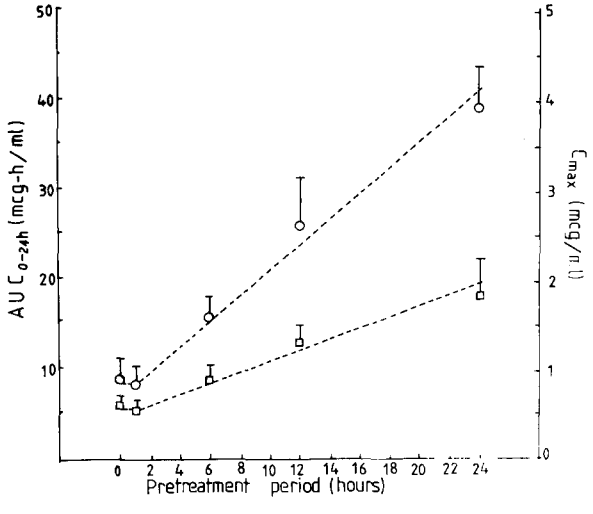

Fig. 2. Relationship between the pretreatment period and the AUC_{0-24h} or C_{max} ($\mu g/\mu l$) values of absorbed piroxicam by using 80% DMSO/PG as the penetration enhancer for pretreatment. (○------○) For AUC_{0-24h}; (□------□) for C_{max}. Each point represents the mean of five rabbits with the standard error.

detectable until after 1 h exposure to DMSO (Sharata et al., 1988). Therefore, the piroxicam may penetrate mainly through the intercellular lamellar lipid pathway. It has been reported that the fluidity of the intercellular lipid increases as a function of DMSO concentration (Khan et al., 1989). We assumed that the intercellular lamellar lipids or intracellular ultrastructure of stratum corneum might be in contact with a greater amount of DMSO and cause more fluidity on increasing the pretreatment period from 1 to 24 h, resulting in the absorption of piroxicam increasing linearly with increase in the pretreatment period.

When the skin was pretreated with 10% oleic acid/PG for 0.5, 1, 6, 12, 18, and 24 h before topical applications of the piroxicam gel, its plasma concentration-time profiles of absorbed piroxicam from 0 to 24 h for each condition and nonpretreatment were measured as shown in Fig. 3. The lag time for 0.5 and 1 h pretreatment was nearly diminished. However, the lag time was 2 h in the nonpretreatment. The AUC_{0-24h} or C_{max} and F_{0-24h} values are shown in Table 1. A specific correlation between the AUC_{0-24h} or C_{max} value of absorbed piroxicam and the pretreatment period was found, as shown in Fig. 4. The AUC_{0-24h} values for 0, 0.5, and 1 h pretreatment increased linearly with increasing pretreatment period. The AUC_{0-24h} value for 1 h pretreatment was 8-fold higher than that of nonpretreatment and was not significantly different from that of 6 or 12 h

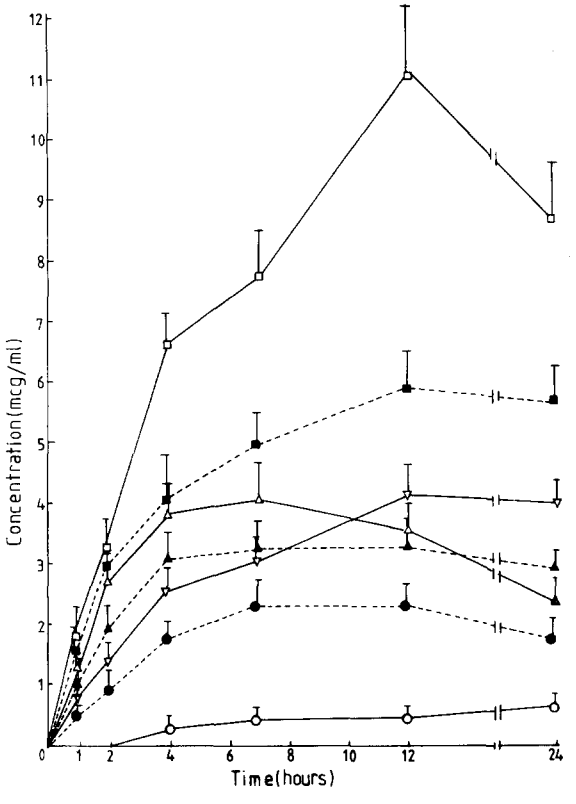

Fig. 3. Effect of pretreatment period on the plasma concentration-time curve of the absorbed piroxicam by using 10% oleic acid/PG as the penetration enhancer for pretreatment. (○———○), Nonpretreatment; (●------●) 0.5 h; (△———△) 1 h; (▽———▽) 6 h; (▲------▲) 12 h; (■------■) 18 h; (□———□) 24 h. Each point represents the mean of five rabbits with the standard error.

pretreatment (ANOVA test, $p > 0.05$). The AUC_{0-24h} or C_{max} values for 12, 18 and 24 h pretreatment increased linearly as the pretreatment period increased. The AUC_{0-24h} value of absorbed piroxicam for 24 h pretreatment was about 22-fold higher than that of nonpretreatment and the absolute bioavailability obtained was 62%. The photomicrograph shown in Fig. 5C indicates that the skin pretreated with 10% oleic acid/PG even for 12 h does not cause appreciable exfoliation of the stratum corneum, but more serious damage and exfoliation were observed for that of 18 h pretreatment as shown in Fig. 5D. Oleic acid mainly modified the non-polar route (Barry et al., 1987) and would partition into the stratum corneum and disrupt the stratum corneum lipid packing and, hence, decrease diffusion resistance to permeants (Golden et al., 1987). In the present study, it is assumed that the stratum corneum lipid packing may gradually be influenced by pretreating the stratum corneum with 10% oleic acid/PG for 0.5 and 1 h, and results in the penetration enhancing effect increasing linearly with increasing pretreatment period. It has been reported that oleic acid may remain in the skin for a considerable time after application to the skin (Barry et al., 1987; Goodman et al., 1988). It has also been reported that rat skin pretreated with 10% oleic acid/PG for 1 h shows the same effect for percutaneous absorption of molsidomine as 6 h pretreatment (Yamada et al., 1987b). It is assumed that oleic acid might remain and saturate in the intercellular lipid lamellar of stratum corneum during 1, 6 or 12 h pretreatment periods and result in a similar fluidity of the lipid and a comparable penetrated enhancing effect for 1, 6 and 12 h pretreatment in the present study. When the pretreatment period is more than 12 h, one condition might appear. That is, the longer the duration of the pretreatment period, the greater will be the extent of exfoliation and damage to the

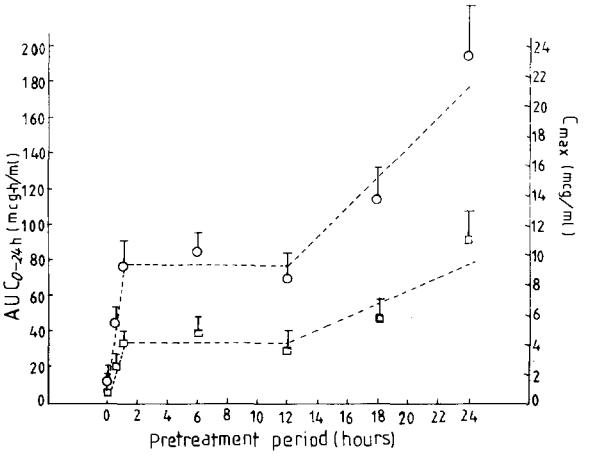

Fig. 4. Relationship between the pretreatment period and the AUC_{0-24h} or C_{max} values of absorbed piroxicam by using 10% oleic acid/PG as the penetration enhancer for pretreatment. (○------○) for AUC_{0-24h}; (□------□) for C_{max}. Each point represents the mean of five rabbits with the standard error.

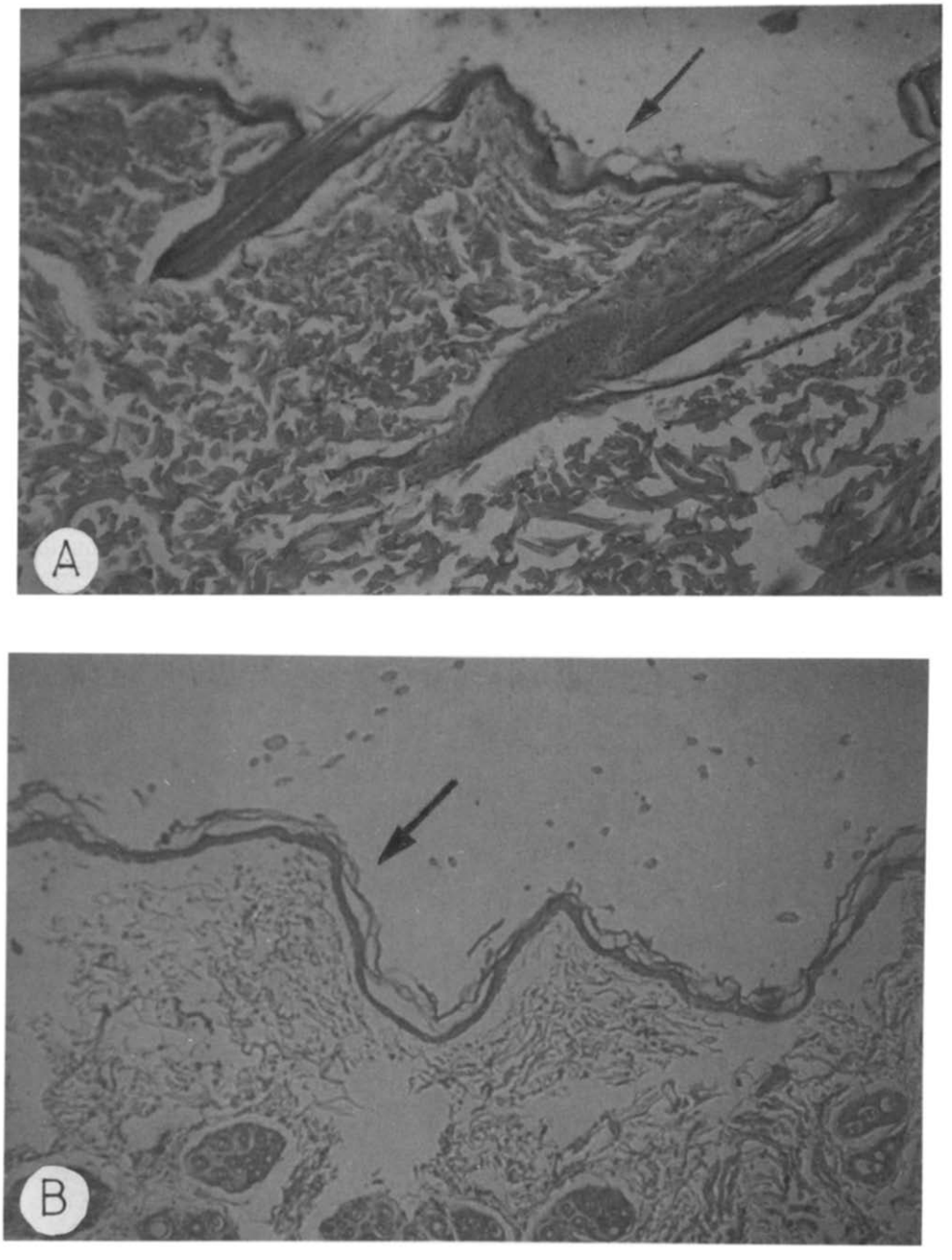

Fig. 5. Stained cross-section of stratum corneum of rabbit skin. Black arrows indicate the stratum corneum; white arrow indicates the exfoliation of stratum corneum or epithelium. (A) Intact skin. (B) Skin treated with 80% DMSO/PG for 24 h. (C) Skin treated with 10% oleic acid/PG for 12 h. (D) Skin treated with 10% oleic acid/PG for 18 h. Bar = 50 μm.

stratum corneum or epithelium. Furthermore, piroxicam will penetrate more extensively into the skin.

In conclusion, the percutaneous absorption of piroxicam gel has been markedly improved by pretreating the skin with 10% oleic acid/PG even

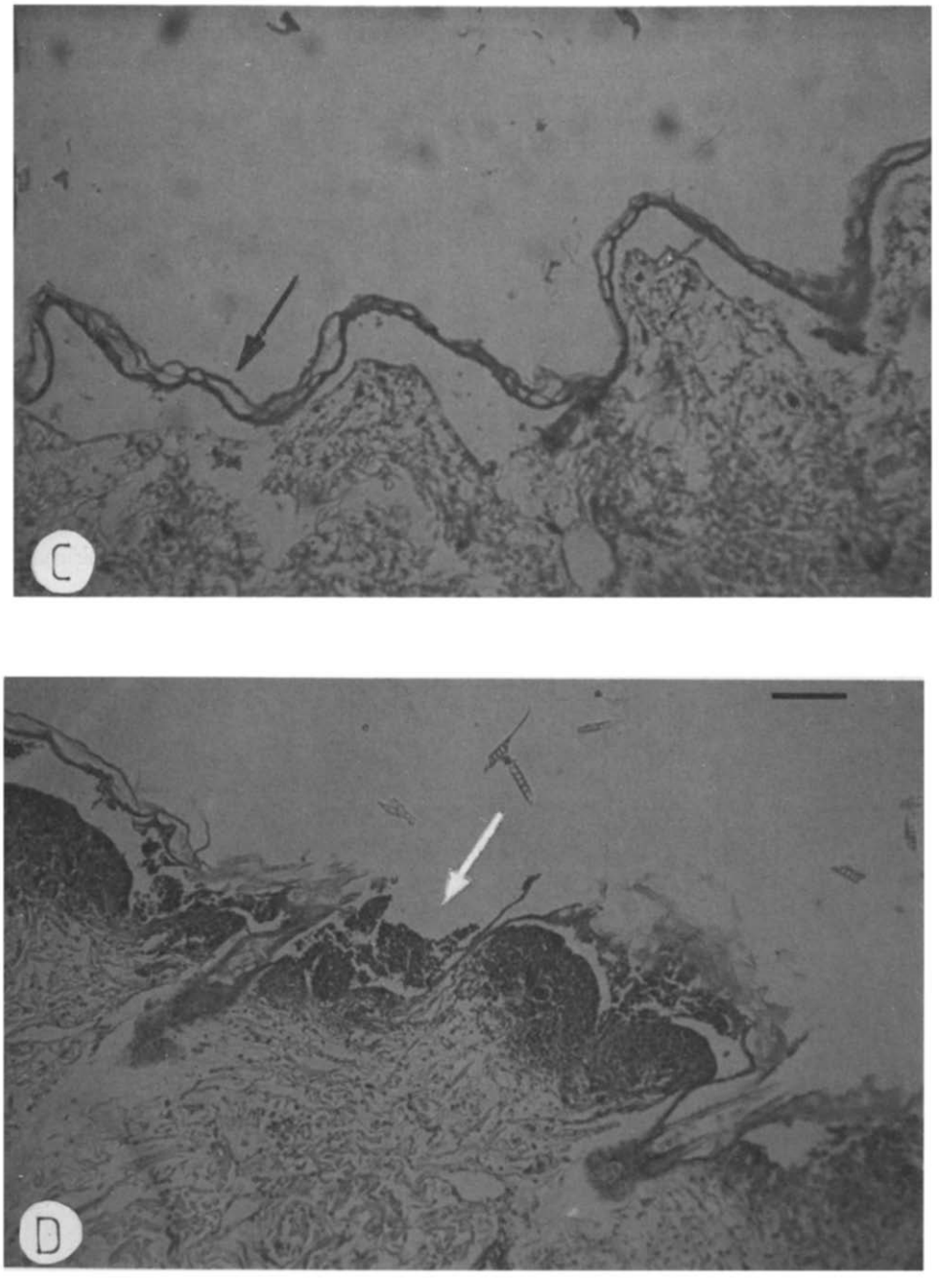

Fig. 5 (c, d).

for 1 h. A specific relationship between ACU_{0-24h} or C_{max} value and the pretreatment period has been demonstrated in this study. Thus, an excellent transdermal drug delivery system for piroxicam gel was developed and could be applied to other drugs or dosage form.

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