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PERCUTANEOUS ABSORPTION OF PIROXICAM FROM FAPG BASE THROUGH RAT SKIN: EFFECTS OF OLEIC ACID AND SATURATED FATTY ACID ADDED TO FAPG BASE

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ABSTRACT

Oleic acid (OA) or / and saturated fatty acid (i.e. lauric , myristic, palmitic and stearic acid) decreased the release of piroxicam from FAPG (fatty alcohol-propylene glycol) base. The reason would be due to the increase in lipophilicity of the base. The released piroxicam was found to be inversely proportional to the apparent viscosity of the ointments containing OA and saturated fatty acid. However, OA or / and the saturated fatty acids enhanced the in vitro skin permeation and the in vivo percutaneous absorption of piroxicam, the enhancing effect was decreased linearly with increasing carbon number of saturated fatty acid from 12 to 18. A useful parameter has been obtained for estimating the properties of the exudation or "bleeding" of the FAPG base . A number of piroxicam FAPG ointments have been selected as an optimal product for less bleeding and more percutaneous absorption of piroxicam than those of controls (containing no oleic acid and saturated fatty acid).

INTRODUCTION

FAPG base, a mixture of fatty alcohol (FA), propylene glycol (PG) and other excipients (1), has been used as a topical vehicle for various corticosteroids (2, 3, 4, 5) and also used to investigate the effect of palmitic acid, stearic acid, cetyl alcohol and OA on the percutaneous absorption of indomethacin (6, 7, 8). OA is an effective penetrating enhancer (9, 10, 11, 12, 13, 14, 15). Saturated fatty acid (i.e. lauric, myristic, palmitic and stearic acid) can be used as the penetrating enhancer (11, 12, 16) or stabilizer to prevent the bleeding of the FAPG base (1, 6). In the present study, we investigated the effect of OA or/and the saturated fatty acid on the percutaneous absorption of drug from the FAPG base. Piroxicam is an non-steroid antiinflammatory analgesic drug. Oral administration of piroxicam is effective for the treatment of rheumatoid arthritis (17, 18); it has an irritational side-effect on gastrointestinal mucosa (19). A few traditional topical ointments of piroxicam have been prepared to prevent this side-effect (20, 21). However, it appears that little or no attention has been paid to the piroxicam FAPG ointment. So, we selected piroxicam as a model drug to investigate the effect of OA or/and saturated fatty acid on the percutaneous absorption of piroxicam FAPG ointment.

MATERIAL AND METHOD

Materials - Piroxicam (Pfizer, U.S.A.), indomethacin (Sumitomo , Japan), stearyl alcohol, stearic acid, palmitic acid, myristic acid, lauric acid, propylene glycol, PEG 400 and oleic acid, diethyl ether (Merk, Germany) were obtained commercially . All other chemicals were of analytical reagent grade. The cellulose membrane, C-110, was purchased from Visking Co., Ltd.

Preparation of piroxicam FAPG ointments - FAPG bases con-

Table 1

Formulae of FAPG ointment (1 % piroxicam)

Composition [a]	Ointment					
	1	2	3	4	5	6
Stearyl alcohol	25	25	20	20	20	20
Propylene glycol	75	70	70	70	70	70
Lauric acid	-	-	5	-	-	-
Myristic acid	-	-	-	5	-	-
Palmitic acid	-	-	-	-	5	-
Stearic acid	-	-	-	-	-	5
Oleic acid	-	5	5	5	5	5

a) mass/ gram

taining 1 % piroxicam were prepared according to the formulae in Table 1. The stearyl alcohol or / and fatty acid, OA were heated at 75 °C; then the piroxicam mixed with PG, and previously heated to the same temperature, was added. The mixture was stirred until it congealed.

In vitro release experiment - The vertical and horizontal type of diffusion cell was similar to the apparatus of the Franz diffusion assembly (22). The Visking seamless cellulose tubing was used as the membrane. An amount of test FAPG sample and phosphate buffer (pH 7.4, 20 ml, containing 20 % PEG 400) were poured into the donor and receptor container of the cell , respectively. The temperature of the solution was held at 37 ± 0.1 °C, with stirring at 700 rpm. Sample solution (0.5 ml) was taken up at an appropriate time and assayed with the spectrophotometer (Hitachi 200-10, Hitachi Seisakusho Co. Ltd) at 365 nm. The volume of the receptor phase was kept constant throughout the release run. The amount of piroxicam released per unit area during 48 h of each piroxicam FAPG ointment was determined.

Measurement of the bleeding degree - According to the in vitro release experiment described above, the equal amount of same

test sample was filled into the donor container of the vertical and horizontal - type diffusion cell. The calculated bleeding degree (B) was obtained as:

$$B = (W_1 - W_2) / W_P \qquad \text{Eq. 1}$$

in which W_1 and W_2 are the amount of piroxicam released during 12 hrs from the vertical and horizontal type diffusion cell, respectively; W_P is the amount of piroxicam in the original test sample. The observed bleeding degree (H) was obtained by measuring the volume of the layer of bleeding liquid from 40 g of the test sample which was contained in a 100 ml graduated beaker for one year at room temperature.

Measurement of partition ratio - FAPG base (5 g) and piroxicam water solution (10 ml, 10 μg /ml) were placed in a glass-stoppered test tube and shaken in a water bath at 60 ± 0.1 °C for 2 h. The tube was removed and left at room temperature in a vertical position for 12 h to allow separation of the two phases and solidification of the fatty alcohol or / and fatty acid phase. The amount of piroxicam in the water phase was determined with a UV spectrophotometer. The partition ratio (w/o) was calculated by comparing the amount of piroxicam in the water phase (w) with that in the fatty alcohol or / and fatty acid phase (o).

Measurement of viscosity - The cone - and - plate viscometer (Brookfield RVT DVII, Brookfield Engineering Laboratories, Inc.) was used to determine the viscosity of piroxicam FAPG ointment at 34 °C. The maximum shear rate was 38.4 s^{-1} with a sweeping period 30 s.

In vitro skin pretreatment experiment - Male rats weighing between 180 and 220 g were used. The hair of the abdominal region was removed with electric hair clippers the day before the experiments. The freshly excised skin was fixed in the diffusion cell and pretreated with each FAPG base (containing

no piroxicam) for 12 h under the conditions in which the lower receptor container of the diffusion cell was filled with the phosphate buffer (pH 7.4, containing 20 % PEG 400) at 37 °C. The skin was then gently swabbed clean 20 times with cotton to remove the residual ointment without damaging the skin. Subsequently, the piroxicam FAPG ointment (1.5 g, 1 % piroxicam FAPG ointment containing no additives) was used as a model drug to penetrate the treated skin under the conditions of the in vitro release experiment described above. The amount of piroxicam penetrated per unit area during 48 h was measured.

In vivo percutaneous absorption experiment - The rat was fixed on its back, and 1 % piroxicam FAPG ointment (4 g) was spread on the dehaired abdominal skin (6 x 3.3 cm^2) with occlusive dressing technique (ODT) (23). Blood samples (0.5 ml) were withdrawn from the carotid artery by puncturing into a syringe at predetermined intervals, and were centrifuged at 3000 rpm for 10 min. The resulting plasma samples were individually subjected to piroxicam content measurement by HPLC. The AUC value of piroxicam was calculated according to the trapezoidal rule.

Assay of piroxicam - The HPLC method for the analysis of piroxicam was described previously (24).

RESULTS AND DISCUSSIONS

A good linear correlation between the piroxicam released per unit area and the root of time for each piroxicam FAPG ointment is shown in Figure 1A. The Higuchi diffusion equation (25, 26) may clearly be used in the suspension type of piroxicam FAPG ointment. The amount of piroxicam released per unit area during 48 h (q_t^r) for each ointment is listed in Table 2 and the relationship among them is indicated in Figure 1B, it was shown that the piroxicam FAPG ointment containing

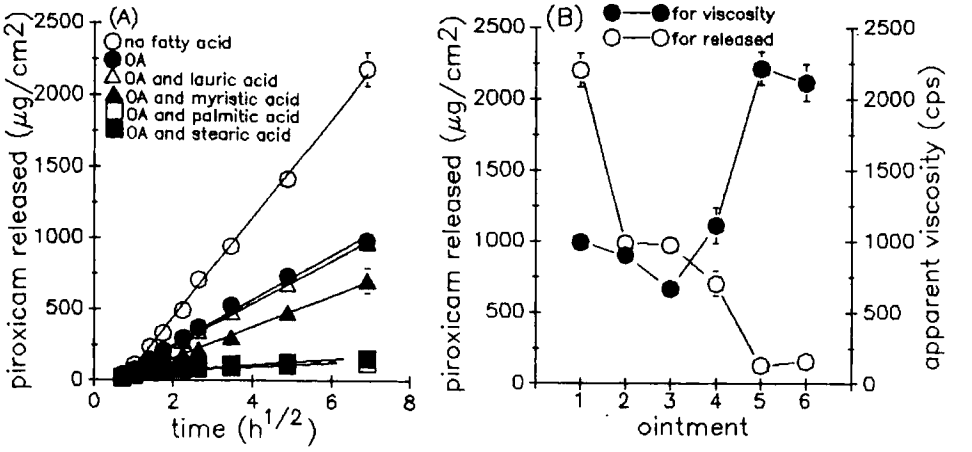

Figure 1

A: effect of OA or/and saturated fatty acid on the release of piroxicam from FAPG base; B: the relationship between the amount of released piroxicam and the apparent viscosity of each piroxicam FAPG ointment. Vertical bar are standard error (n = 5).

Table 2

The value of q_i^r, Q_i^p and AUC_{ob} for each piroxicam FAPG ointment

Ointment	q_i^r μg cm^{-2}	Q_i^p μg cm^{-2}	AUC_{ob} μg h ml^{-1}
1	2189.4 ±117.2	35.3 ±3.4	18.5 ±3.4
2	983.7 ±52.8	229.5 ±23.1	85.2 ±10.6
3	967.9 ±10.3	710.2 ±66.8	315.1 ±36.6
4	701.7 ±90.0	460.3 ±60.3	212.4 ±26.2
5	125.6 ±12.3	292.8 ±42.7	82.7 ±10.2
6	156.0 ±16.8	200.9 ±46.0	65.8 ±8.4

q_i^r: piroxicam released per unit area; Q_i^p : piroxicam penetrated through pretreated skin; AUC_{ob}: the observed value of AUC of percutaneous absorption of piroxicam. Data are means ± S.E. (n = 5)

Table 3

Apparent partition ratio of piroxicam between water and FAPG base (water/base)

	Base of ointment					
	1	2	3	4	5	6
Partition ratio	2.142	0.781	0.429	0.342	0.354	0.363
S D	0.783	0.134	0.107	0.015	0.019	0.059

Each value represents the mean of three experiments.

Table 4

Apparent viscosity of piroxicam FAPG ointment at 34 °C

	Ointment					
	1	2	3	4	5	6
Apparent viscosity (cps)	992	896	666	1109	2214	2118
S E	58	50	12	123	113	129

Each value represents the mean of five experiments.

no OA and saturated fatty acid had the greatest amount of released piroxicam. According to the result of experiment of partition ratio (w/o) (Table 3), such ratio is greatest in the FAPG ointment containing no OA and saturated fatty acid (No. 1 base); less marked for that of containing OA alone (No. 2 base); and the still less marked for that of containing of OA and saturated fatty acids (No. 3, 4, 5 and 6 base). It is assumed that OA added to the FAPG base increased the lipophilicity of the base and then decreased the amount of the released piroxicam; further addition of the saturated fatty acid will potentiate this effect. Table 4 and Figure 1B show the apparent viscosity and its relationship among each piroxicam FAPG ointment. It was shown that the viscosity of the piroxicam FAPG ointment containing OA and palmitic acid (No. 5

ointment) was the most viscous of all. A similar result has been reported that the indomethacin FAPG ointment containing palmitic acid was more viscous than that of stearic acid or no saturated fatty acid (6). Futhermore, the released piroxicam was found to be inversely proportional to the viscosity of the piroxicam FAPG ointment containing a particular saturated fatty acid (Figure 1B). It is assumed that because the piroxicam in the FAPG ointment containing saturated fatty acid (No. 3, 4, 5 and 6 ointment) had a similar partition ratio (ANOVA test, $p > 0.05$) as shown in Table 3, piroxicam then having a similar thermodynamic activity in these base, thereby the released piroxicam is proportional to the diffusion coefficient according to the Higuchi diffusion equation (25, 26) and then is inversely proportional to the viscosity according to the Einstein Stoke Equation (27).

According to the in vitro skin pretreatment experiment, the profile of the piroxicam penetrated per unit area versus time from 0 to 48 h for each formula is shown in Figure 2A; the piroxicam penetrated per unit area during 48 h (Q_i") are listed in Table 2. The rat skin pretreated with OA (in FAPG base) increased the extent of penetration of piroxicam through the skin, futher additon of the saturated fatty acid will potentiate this effect except stearic acid. Especially, pretreatment with OA and lauric acid (in FAPG base) was about 3-fold higher than that of ointment containing OA alone and was about 20-fold higher than that of controls (no OA and saturated fatty acid). The pretreatment enhancing effect of saturated fatty acid decreased linearly with increasing carbon number of the saturated fatty acid from 12 to 18 ($r=0.979$). Similar results have been reported for the percutaneous absorption of naloxone (16).

According to the in vivo percutaneous absorption experiment, the plasma piroxicam concentration-time curve of piro-

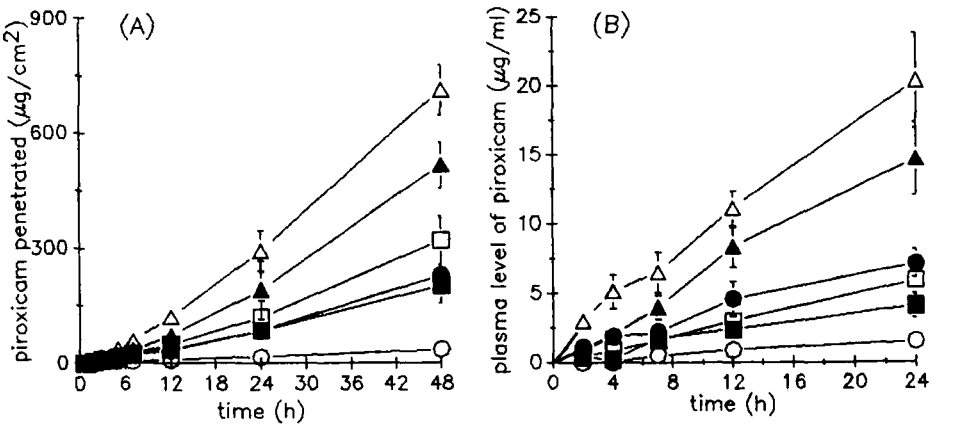

Figure 2

A: the in vitro piroxicam penetrated-time curve for skin pretreated with each FAPG base containing OA or/and saturated fatty acid or none of these; B: the in vivo plasma concentration-time curve of piroxicam for each piroxicam FAPG ointment. (○), no fatty acid; (●), OA alone; (△), OA and lauric acid; (▲), OA and myristic acid; (□), OA and palmitic acid; (■), OA and stearic acid. Vertical bar are standard errors (n = 5).

xicam absorbed from 0 to 24 h and the observed AUC value (AUC_{obs}) for each formula are shown in Figure 2B and Table 2, respectively. According to the result, the AUC_{obs} value for the piroxicam FAPG ointment containing OA and lauric acid was about 17-fold greater than that of controls. OA or lauric acid has also been shown to increase the percutaneous absorption of many drugs. A linear correlation (r= 0.983) was found between the in vitro penetrated piroxicam (Q_t^n) and the in vivo percutaneous absorption of piroxicam (AUC_{obs}) (Figure 3).

The calculated bleeding degree (B), which was obtained from Eq.1, and the observed bleeding degree (H) are listed in the Table 5; it was found that the piroxicam FAPG ointment containing OA and lauric acid (No. 3 ointment) had the largest value of calculated and observed bleeding degree; less marked for that of containing OA alone (No. 2 ointment); and still

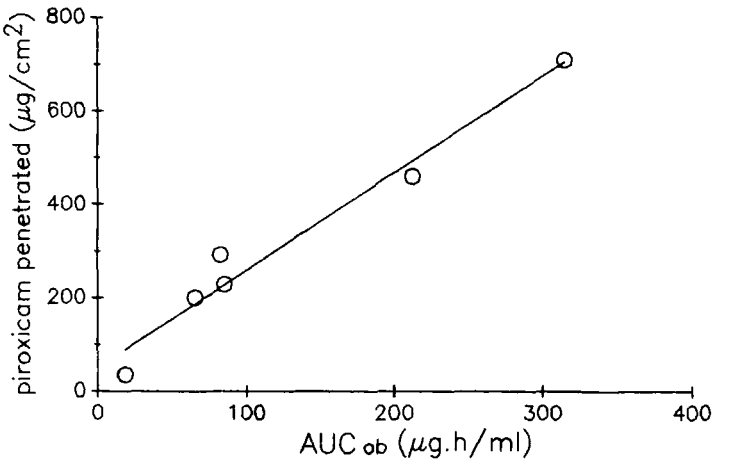

Figure 3

Scatter diagram of the values of AUC_{ob} and Q_t^P (Table 2).

Table 5

Calculated and observed bleeding degree for each piroxicam FAPG ointment

	ointment					
	1	2	3	4	5	6
B (%)	3.64 ±0.28	4.20 ±0.24	9.23 ±0.89	1.51 ±0.32	1.15 ±0.25	1.53 ±0.34
H (ml)	0	6.22 ±0.86	10.47 ±1.44	0	0	0

B: calculated bleeding degree; H: observed bleeding degree
Data are means ± S.E. (n = 5)

less marked for that of controls (No. 1 ointment) or other saturated fatty acids (No. 4, 5 and 6 ointment) in which the calculated bleeding degrees were below 3.64 and no visible layering of liquid was found. By this, it can be said that the calculated bleeding degree can be a useful comparable parameter to estimate the observed bleeding degree of piroxicam

FAPG ointment after prolonging storage. In addition, we expected that the piroxicam FAPG ointment containing myristic, palmitic or stearic acid will be more stable for the less calculated bleeding degree than that of the controls.

In conclusion, OA or / and saturated fatty acid added to the piroxicam FAPG ointment increased the lipophilicity of the base and decreased the piroxicam released from the base. The released piroxicam was found to be inversely proportional to the apparent viscosity of the ointment containing OA and saturated fatty acid. Pretreating the skin with OA and saturated fatty acids (in FAPG base) enhanced the permeability of the skin to the piroxicam; the enhancing effect decreased linearly as the carbon number of saturated fatty acid increased from 12. to 18. A linear correlation was presented between the in vitro piroxicam peretrated through treated skin and the in vivo percutaneous absorption of piroxicam from FAPG base. A useful parameter has been obtained for estimating the properties of bleeding of the FAPG base. The piroxicam FAPG ointment containing OA and saturated fatty acid such as myristic, palmitic or stearic acid has been selected as an optimal product for less bleeding and more percutaneous absorption of piroxicam than that of controls.

References

1. M. Katz, Atherton, Calit. and H. M. Neiman, U. S. Patent 3592930(1971).
2. M. F. Coldman, L. Lockerbie and E. A. Laws, Br. J. Derm., 85, 573-576(1971).
3. M. Henry, Curr. Med. Res. Opin., 2, 27-31(1974).
4. J. Ostrenga, J. Naleblian, B. Poulsen, B. Ferrela, N. Mueller and S. Shastri, J. Invest. Dermatol., 56, 392 (1971).

5. E. L. Rhodes, W. L. Morison, G. B. Skinner, D. W. Borrett and M. Stoppard, Curr. Med. Res. Opin., 1, 87-92(1972).
6. F. kaiho, H. Nomura, E. Makabe and Y. Kato, Chem. Pharm. Bull., 35, 2928-2934(1987).
7. F. Kaiho, Y. Takahashi, R. Ichihara, A. Watanabe, T. Yamashita and Y. Kato, Yakugaku Zasshi, 106, 47-53(1986).
8. H. Nomura, F. Kaiho, Y. Sugimoto, Y. Miyashita, M. Dohi and Y. Kato, Chem. Pharm. Bull., 38, 1421-1424(1990).
9. B. W. Barry and S. L. Bennett, J. Pharm. Pharmacol., 39, 535-546(1987).
10. E. R. Cooper, E. W. Merritt and R. L. Smith, J. Pharm. Sci ., 74, 688-689(1985).
11. M. Yamada and Y. Uda, Chem. Pharm. Bull., 35, 3390-3398 (1987).
12. P. G. Green, R. H. Guy, and J. Hadgraft, Int. J. Pharm., 48, 103-111(1988).
13. E. M. Niazy, Int. J. Pharm., 67, 97-100(1991).
14. T. Seki, T. Kawaguchi, K. Sugibayashi, K. Juni and Y. Morimoto, Int. J. Pharm., 57, 73-75(1989)
15. M. Yamada, Y. Uda and Y. Tanigawara, Chem. Pharm. Bull., 35, 3399-3406(1987).
16. B. J. Aungst, N. J. Rogers and E. Shefter, Int. J. Pharm., 33, 225-234(1986).
17. D. C. Hobbs and T. M. Twomey, J. Clin. Pharmacol., 19, 270-281(1979) .
18. R. K. Verbeeck, J. L. Blackburn and G. R. Loewen, Clin. Pharmacokinet., 8, 297-331(1983).
19. P. Schiantarelli and S. Cadel, Arzneim.-Forsch./ Drug Res. ,31, 87-92(1981).
20. Y. H. Tsai, L. R. Hsu and S. I. Naito, Int. J. Pharm., 24, 61-78(1985).
21. P. Dallas, M. B. Sideman, J. Polak and F. M. Plakogiannis, Drug Develop. & Ind. Pharm., 13, 1371-1397(1987).

22. Y. W. Chien, Drug Develop. & Ind. Pharm., 13, 589-651 (1987).
23. S. I. Naito and Y. H. Tsai, Int. J. Pharm., 8, 263-276 (1981).
24. L. R. Hsu, Y. H. Tsai and Y. B. Huang, Int. J. Pharm., 71, 193-200(1991).
25. M. Katz and Z. I. Shaikh, J. Pharm. Sci., 54, 591-594 (1965).
26. B. Idson, J. Pharm. Sci., 64, 901-924(1975).
27. W. I. Higuchi, J. Pharm. Sci., 56, 315-324(1967).