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Topical absorption of piroxicam from organogels—in vitro and in vivo correlations

T. Pénzes [a], G. Blazsó [b], Z. Aigner [a], G. Falkay [b], I. Erős [a,*]

[a] *Department of Pharmaceutical Technology, University of Szeged, Eötvös u. 6, Szeged H-6720, Hungary*
[b] *Department of Pharmacology and Biopharmacy, University of Szeged, Eötvös u. 6, Szeged H-6720, Hungary*

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Abstract

In view of their good skin tolerability, glyceryl fatty acid esters were used as organogelators, and their effects in the topical penetration of piroxicam (Px) were investigated. The in vivo skin penetration was evaluated by measuring the anti-inflammatory effect in rats, where we found that Px incorporated into glyceryl fatty acid ester organogels exhibited a significantly greater inhibition of oedema than that of the placebo control either when applied locally ($p < 0.001$), or via transdermal absorption ($p < 0.01$ and <0.05, respectively). As the Px concentration was increased, the extent of oedema inhibition rose in accordance with a power law. Comparisons with traditional galenic organogels and a marketed product revealed that the relative biological availability of Px was better from glyceryl fatty acid ester organogels, except when calculated for D1 versus T2 and T3. In order to predict the extent of in vivo skin absorption, we measured the penetration coefficient and the in vitro penetration. In accordance with theory, the extent of in vivo oedema inhibition increased as $P_{oct/w}$ increased, and maximum inhibition was observed at $\log P = 2.0211$. However, the in vitro penetration through a synthetic membrane did not correlate with the in vivo results, the reason for which might be the different natures of the model barriers.

Keywords: Organogel; Piroxicam; Penetration; Bioavailability

1. Introduction

Organogels are gels based on non-aqueous liquids, which have been mentioned in various pharmacopoeias as useful topical deliveries for lipophilic drugs (Martin, 1993). They not only exert a local effect but also are capable of achieving a systemic effect via transdermal absorption, when their lipophilic nature and occlusive effect are potentiated by the presence of a penetration enhancer.

Since the discovery of simple gelator molecules, organogels have received great attention. These novel molecules can be used in small quantities, without further additives, thereby resulting in more biocompatible products (Murdan et al., 1999a; Gronwald et al., 2002).

* Corresponding author. Tel.: +36 62 545571; fax: +36 62 545571.
E-mail address: eros@pharm.u-szeged.hu (I. Erős).

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Besides the synthesis of new gelators, organogel research focuses on their applicability in various fields. A number of new-generation organogels have recently been described as effective vehicles for transdermal delivery (Lawrence and Rees, 2000; Shchipunov, 2001), and they have also proved useful as immune adjuvants (Murdan et al., 1999b,c).

Although the penetration-enhancing ability of fatty acid esters has been described by several authors, topical semisolid formulations include them generally as stabilizers or emulsifier additives. The mechanism of action of penetration enhancement is considered to be the disorganization of the lipid structures in the stratum corneum by packing alongside the lipids and fluidization of the head-group region (Walker and Smith, 1996; Cornwell et al., 1998; Schreier et al., 2000; Gwak and Chun, 2002). A further interesting property of these chemicals is their ability to act as organogelators for various liquids (Murdan et al., 1999d).

In the present study, two-component organogels based on liquid glyceryl fatty acid esters and including solid glyceryl fatty acid esters as organogelators were tested for their ability to enhance the skin penetration of piroxicam (Px), a non-steroidal anti-inflammatory drug (NSAID), which is indicated in inflammatory diseases, articular complaints, rheumatoid arthritis and osteoarthritis. When these conditions are to be treated topically, there is a need for a vehicle which ensures the deep skin penetration of Px. Various vehicles and skin-pretreatment techniques are known with which the topical availability of Px can be increased (Tsai et al., 1985; Santoyo et al., 1995; Shin et al., 1999; Shin et al., 2000; Dalmora et al., 2001). In the present study, the anti-inflammatory effects of glyceryl fatty acid ester organogels were compared with those of two extensively used traditional organogels and a marketed product. We also attempted to predict the extent of in vivo penetration via in vitro penetration data, and to establish in vitro–in vivo correlations.

2. Materials and methods

2.1. Materials

Glyceryl mono- and distearate SE (F1), glyceryl monostearate/palmitate (F2) and glyceryl stearate (F3) were from Goldschmidt, Germany; Mygliol® 812 (fractionated coconut oil, glyceryl tricaprylate/caprate) was from Hüls, Germany; isopropyl myristate, disodium hydrogenphosphate and citric acid were from Merck, Germany, and piroxicam was from Egis, Hungary.

Table 1 summarizes the compositions used in this study.

2.2. Sample preparation

Solid fatty acid esters (13%, w/w) were dispersed in the oil (87%, w/w) at 70 °C on a water bath. The transparent, liquid-like melted mixture (sol state) was allowed to cool down to 25 °C under vigorous stirring (120 rpm), during which crystals were formed, which built up a three-dimensional network through the oil (gel state). Px (1%, w/w) was then dispersed in the organogels.

Table 1
Compositions of formulations

	Organogel base	Consistency modifier	Emulsifier
Developed organogels			
D1		Glyceryl mono- and distearate SE (F1)	
D2	Mygliol® 812	Glyceryl monostearate palmitate (F2)	
D3		Glyceryl stearate (F3)	
Traditional organogels			
T1[a]	White petrolatum	Cetostearyl alcohol	Lanolin alcohols
T2[b]	Liquid paraffin	Colloidal silica	–
T3		Marketed product	

[a] Hungarian Pharmacopoeia (VIIth ed.).
[b] Hungarian National Formulary (VIth ed.).

2.3. Investigation of solubility in phosphate buffer

An excess amount of Px was added to phosphate buffer (pH 5.4 ± 0.1) containing 0.5% (w/w) fatty acid ester. The mixture was shaken for 7 days at 25 °C, the supersaturated solution was then filtered, and the Px content of the saturated solution was determined with a Unicam UV2 UV–vis spectrometer (Unicam, UK) at 356 nm.

2.4. Determination of partition and penetration coefficient

The water and the *n*-octanol were saturated with each other, and 0.5% (w/w) fatty acid ester with 0.5% (w/w) Px was the added to the separated phases. The mixtures were shaken for 7 days at 25 °C, and c_{oct} and c_w were then determined spectrophotometrically. The permeability was estimated via the equation derived by Potts and Guy (1992):

$$\log\ Kp = -2.7 + 0.71\ \log\ P - 0.0061\,MW \qquad (1)$$

where *K*p is the penetration coefficient, *P* is the *n*-octanol/water partition and MW is the molecular weight.

2.5. In vitro penetration study

To model the percutaneous absorption in vitro, a Franz vertical diffusion cell (Hanson Research, USA) was used. The samples were placed on a cellulose acetate membrane (3 μm pore size, Sartorius, Germany), which was soaked in isopropyl myristate in order to mimic a lipophilic barrier such as the stratum corneum. The acceptor phase was phosphate buffer (pH 5.4 ± 0.1), thermostated at 32 ± 1 °C and stirred with a magnetic bar (450 rpm). At predetermined intervals, 0.75 ml of sample was taken from the acceptor phase and replaced with fresh buffer solution. The Px content was determined spectophotometrically (Helios α® UV–vis spectrophotometer, Thermo Electron, Germany).

The accumulated drug amount penetrated through the unit diffusion surface (*Q*) was calculated and plotted versus time. The steady-state flux of Px (J_s) was estimated from the slope of the linear portion of the penetration curves and expressed as:

$$J_s = \frac{V}{A}\left(\frac{dc}{dt}\right) \qquad (2)$$

where *V* is the acceptor volume, *A* is the diffusion surface area, *c* is the Px concentration in the acceptor phase and *t* is time.

2.6. In vivo anti-inflammatory effect

Experiments were approved by the Animal Ethics Committee of the University of Szeged, Hungary (IV/4316-7/2002).

Female Sprague-Dawley rats (180–200 g) were assigned to weight-balanced groups ($n = 6$ in the first experiment, $n = 8$ in the second experiment). All measurements were performed at 24 ± 1 °C in an air-conditioned room. The experimental groups received the different formulations, while the control group was treated with placebo only. In the first experiment, 150 mg of the different formulations were rubbed gently into the right hind paw (whole hairless skin of legs). The animals were anaesthetized with 400 mg/kg chloral hydrate i.p. in order to prevent the adsorption of the applied organogels by sawdust. The anaesthesia provided sufficient time (∼1 h) for the complete absorption of the samples. In the second experiment, 300 mg of the different formulations were spread over the depilated (Veet®, Reckitt Benckiser, France) dorsal skin (15 cm^2). After 1 h, 0.1 ml of a 0.5% carrageenan suspension (Viscarin®, Marine Colloids, USA) was injected into the subplantar area of the right hind paw. The left paw, used as control, was treated with physiological saline solution without carrageenan (Gábor, 2000). Paw volume was measured with a plethysmometer (Hugo Sachs Elektronik, Germany) 5 h after the carrageenan injection. The degree of paw swelling was calculated as:

$$\text{Swelling}\,(\%) = \frac{V_i - V}{V} \times 100 \qquad (3)$$

where V_i is the volume of the carrageenan-treated paw, *V* is that of the non-treated paw.

On the basis of Eq. (3), the percentage oedema inhibition was calculated as:

$$\text{Inhibition}\,(\%) = \left(1 - \frac{\text{swelling}_{\text{treated}}}{\text{swelling}_{\text{control}}}\right) \times 100 \qquad (4)$$

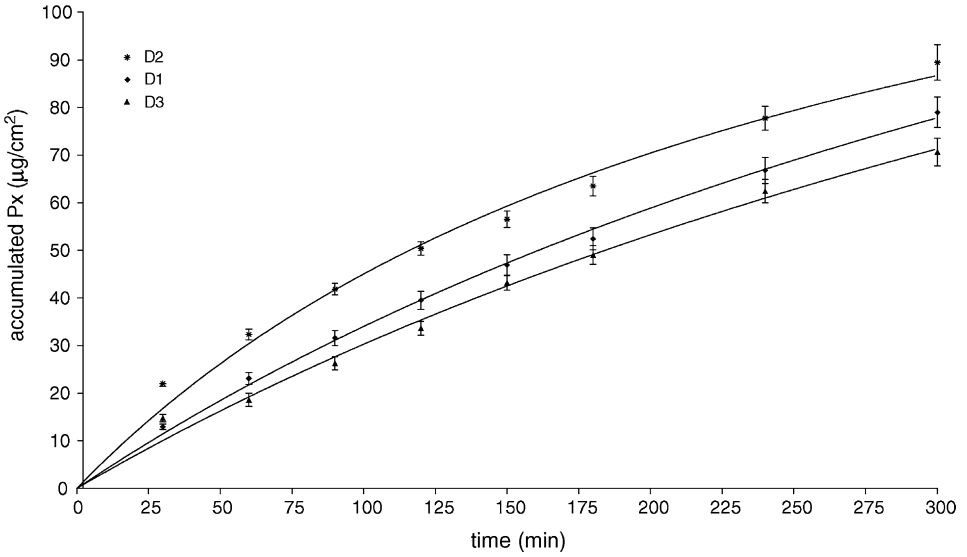

Fig. 1. Effects of glyceryl fatty esters on in vitro penetration of Px ($n = 5$, ±S.E.).

where $\text{swelling}_{\text{treated}}$ is the mean value observed in the treated group, and $\text{swelling}_{\text{tcontrol}}$ is the mean value observed in the control group.

2.7. Data analysis

Statistical analysis was performed by one-way ANOVA, followed by Neumann–Keuls's test, at a significance level of $p < 0.05$ (GraphPad 4.0).

The relative bioavailability (RBA) as regards the systemic effect was calculated as:

$$\text{RBA} = \frac{\text{inhibition\%}_{\text{D}}}{\text{inhibition\%}_{\text{T}}} \quad (5)$$

where $\text{inhibition}_{\text{D}}$ is the percentage oedema inhibition for the different D samples and $\text{inhibition}_{\text{T}}$ is the percentage oedema inhibition for the different T samples.

3. Results

3.1. In vitro approach for prediction of skin penetration

The cumulative amounts of Px at different diffusion times are shown in Fig. 1, and penetration rate constant (k_{p}) and steady-state flux (J_{s}) are presented in Table 2. A slightly higher amount of Px penetrated from D2, and both the k_{p} and J_{s} were also higher as compared with D1 and D3. However, statistically there were no significant differences between the in vitro penetration profiles; according to this prediction, F1-3 will have similar effect on the penetration of Px in vivo.

There is considerable literature evidence that skin penetration can be predicted in vitro via $\log P$, since the K_{p} correlates with $\log P$ (Eq. (1)). A good potency of penetration is most probable when $\log P \sim 2$ (Beetge et al., 2000; Hadgraft and Valenta, 2000; Hadgraft et al., 2000; Yamashita and Hashida, 2003). From the $\log P$ values in Table 3, the sequence of skin absorption should be D2 > D3 > D1.

3.2. In vivo anti-inflammatory effect

When Px was applied locally for pretreatment of the carrageenan-treated area (150 mg of 1% sample), D1

Table 2
Parametes of Px penetration through a lipophilized cellulose membrane ($n = 5$; ± S.E.)

	$Q_{t=300\,s}$ (µg/cm²)	k_{p} (ng/cm²/h)[a]	J_{s} (µg/cm²/h)
D1	78.97 ± 3.21	240.2 ± 79.8	1.5911
D2	89.43 ± 3.71	246.1 ± 9.6	1.6302
D3	70.64 ± 2.88	220.1 ± 9.2	1.4579

[a] Slope of the cumulative drug plotted versus square root time.

Table 3
Effects of glyceryl fatty acid esters on physichochemical properties of Px

	c_{oct} (μg/ml)	c_w (μg/ml)	P	log P	log K_p
Px	0.300	0.017	17.647	1.2466	−3.8364
F1	0.348	0.222	1.568	0.1953	−4.5828
F2	0.210	0.002	105	2.0211	−3.2856
F3	0.260	0.039	6.667	0.8239	−4.1366

resulted in 48.6%, and D2 in 59.4% oedema inhibition (Fig. 2A). As compared with the control group treated with placebo, both samples proved effective ($p < 0.01$), with no significant difference between them. Additionally, Px pretreatment of the dorsal skin (300 mg of 1% sample) inhibited the acute formation of carrageenan-induced paw oedema (Fig. 2B), which indicated a systemic anti-inflammatory effect via transdermal absorption. As compared with the control group, the oedema volume was significantly reduced by both D1 ($p < 0.01$) and D2 ($p < 0.05$). However, the oedema-inhibiting ability of D1 (27.2%) was slight relative to that of D2 (50.3%).

3.3. Dose–response relationship

In order to investigate the dose–response relationship, various concentrations of Px (0.03, 0.1, 0.3, 1 and 3%, w/w) were incorporated into D2 and the systemic oedema-inhibiting effects were measured (Fig. 3). Within the concentration range examined, the degree of in vivo oedema inhibition increased with increasing Px dose in accordance with a power law distribution ($R = 0.8613$).

3.4. Effect of vehicle on bioavailability of piroxicam

The anti-inflammatory effects of glyceyl fatty acid organogels were compared with the one of a commercially available product, and with those of two widely-applied lipophilic vehicles, which are indicated for drug delivery in order to treat rheumatic, inflamed areas (Table 4). When used locally, D1 and D2 were more effective than T1 ($p < 0.001$), but they did not exhibit a significant difference as compared with T2 and T3.

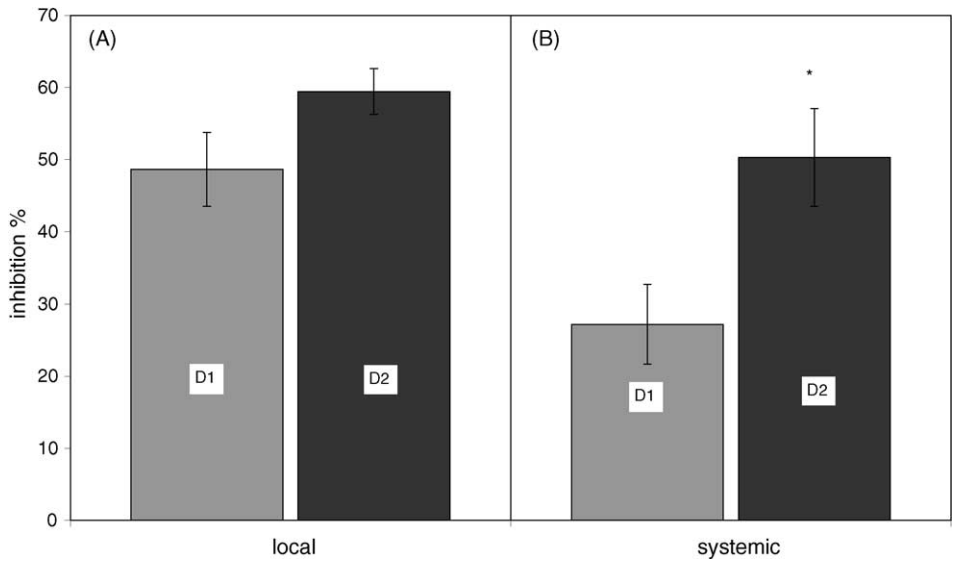

Fig. 2. (A) Comparison of extents of inhibition of paw oedema following administration of different organogels to the paw ($n = 6$); (B) administration of different organogels to the dorsal surface ($n = 8$).

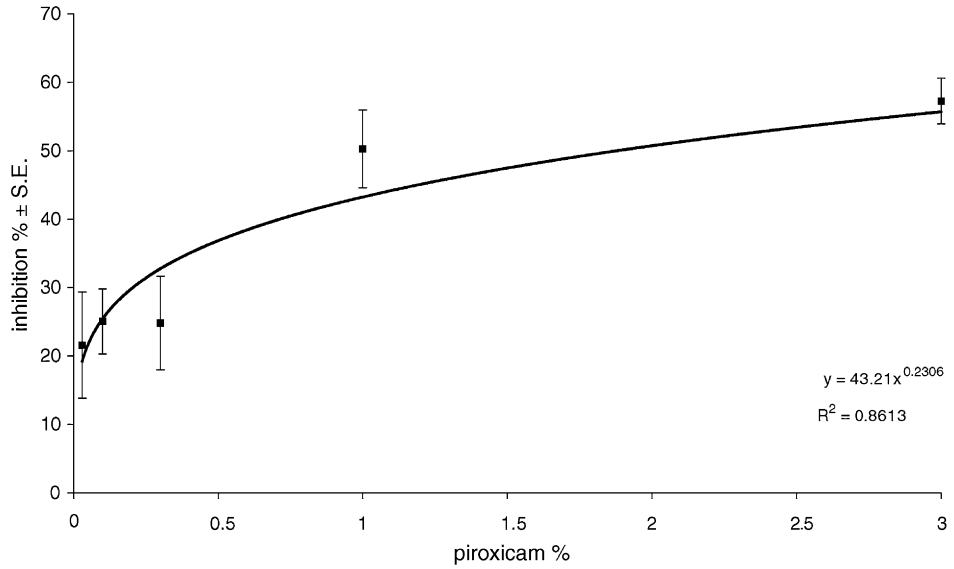

Fig. 3. Dose–response relationship for Px incorporated into D2 organogel ($n = 6$, ±S.E.).

As concerns the systemic effect, D2 was more effective than T1 ($p < 0.001$) and T2 ($p < 0.05$), but there was no significant difference between D2 and T3 or between D1 and T1-2. The in vivo experiments revealed that the RBA of D2 was better than those of T1-3 (3.51; 1.81; 1.24), while the RBA of D1 was better only than that of T1 (1.89; 0.98; 0.67).

3.5. In vitro–in vivo correlation

As mentioned earlier, the optimum log *P* for NSAIDs is ∼2. Below this value the absorption rate increases, while above it the absorption rate decreases (Goosen et al., 1998). From the log *P* values calculated, the sequence of skin absorption should be D2 > D1. This is confirmed by our in vivo data because log *P* for Px was closer to the optimum (2.0211) in the presence of F2, and the skin penetration of Px was better from D2.

However, a different tendency was observed among the organogels as regards their in vitro penetration and in vivo effects. In vitro, there was no significant difference between the D samples, while in vivo, D2 proved to be better than D1 regarding to both local and systemic oedema inhibition effects.

4. Discussion

The in vitro penetration profile of Px from glyceryl fatty acid ester organogels has been described by the Higuchi equation, with good regression coefficients. This model describes drug release as a diffusion process based in the Fick's law, square root time dependent.

Table 4
Anti-inflammatory effect of Px incorporated in different vehicles

	Local		Systemic	
	Swelling (%)	Inhibition (%)	Swelling (%)	Inhibition (%)
D1	25.70 ± 2.2	48.64 ± 5.1	53.113 ± 5.2	27.17 ± 5.5
D2	20.31 ± 1.4	59.40 ± 3.2	36.278 ± 4.1	50.25 ± 7.2
T1	42.87 ± 3.1	12.5 ± 7.1	68.810 ± 12.5	14.33 ± 5.7
T2	31.82 ± 2.9	36.39 ± 6.8	58.388 ± 5.5	27.59 ± 6.8
T3	24.17 ± 2.3	51.76 ± 3.7	43.377 ± 4.9	40.52 ± 5.2

During in vitro penetration, glyceryl fatty acid esters might influence the following features: (1) the partition between the vehicle and the lipophilic layer; (2) the lipophilic/hydrophilic partition between the lipophilized membrane and the acceptor phase; and (3) the solubility of the poorly water-soluble Px in the acceptor phase. In order to ascertain which physicochemical properties govern the in vitro penetration, we investigated the influence of the fatty acid esters on the solubility in phosphate buffer and hydrophilic/lipophilic partition of Px. Whereas F1 and F3 increased the solubility in the acceptor compartment (4.47 and 2.65 times), F2 slightly decreased it (0.74 times). Similar changes in c_w occurred during the determination of partition, and the *n*-octanol/water partition of Px was consequently increased by F2, but decreased by F1 and F3. Different solubility values did not result in significantly different *Q* values, probably because the lipophilic–hydrophilic characters of F1 and F3 were not favourable for diffusion through a lipophilized membrane. In contrast, the more lipophilic F2 might have favoured the penetration, but it did not increase the solubility as F1 and F3 did.

In this study, it was found that D1 and D2 significantly inhibited the acute formation of carragenan-induced paw oedema. The reason for the difference between D1 and D2 is assumed to be the more lipophilic character of F2, which promoted the diffusivity of Px into the skin, and consequently the efficacy of oedema inhibition.

The log *P* values of D1 and D2 correlated with the systemic oedema inhibition effect, whereas the *Q* values did not. The reason for the in vitro penetration and in vivo absorption differences could stem from the different natures of the model barriers used. Cellulose acetate soaked in isopropyl myristate is an inert membrane in vitro, which interacts neither with the active agent nor with the vehicle. Thus, this method models the vehicle/stratum corneum and the stratum corneum/dermis partitions. However, in vivo absorption is more complex because penetration enhancers such as glyceryl fatty acids can interact with the multilamellar lipid matrix of the stratum corneum; the skin is therefore regarded as an active barrier. Accordingly, conclusions must be drawn carefully from comparisons between the results of application of a synthetic barrier in vitro for the prediction of in vivo skin penetration.

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