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Effect of vehicles and penetration enhancers on the *in vitro* percutaneous absorption of celecoxib through human skin

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The aim of this study was the comparison of three different formulations (gel, o/w emulsion, oleagenous cream) and two penetration enhancers (oleic acid and menthol) as vehicle systems for celecoxib in respect of release and penetration through excised human skin *in vitro*. The influence of the vehicle on the release rate was studied *in vitro* using a cellulose acetate membrane. The release rate could be increased by up to 6.5 and 2.5 times with gel and o/w emulsion compared to oleagenous cream respectively. Further *in vitro* penetration measurements using human skin on Franz diffusion cells were performed with and without oleic acid and menthol as enhancers. It was shown that the penetration rate is strongly dependent upon the enhancer type and concentration but not on the vehicle itself and could be increased by 48% when 5% oleic acid was used in oleagenous cream. In all formulations tested, celecoxib was released and penetrated into human skin more quickly and to a greater extent from the gel formulations. There is no topical formulation available of celecoxib and its penetration properties through human skin have not been investigated. Since celecoxib creates some gastrointestinal disturbances, topical formulations of celecoxib preferably in gel form including 5% oleic acid could be suggested as an alternative.

1. Introduction

Celecoxib is a specific COX-2 inhibitor which is used as a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic activity. Its oral administration has been associated with a number of gastrointestinal (GI) disorders. Compared to other NSAIDs, celecoxib, being a specific COX-2 inhibitor, has been found to have less ulcerogenic activity, but it still has a significant potented [1].

During the past few years, skin has been shown to be a suitable delivery route for drugs formulated topically [2]. Researchers have been trying to overcome GI side effects by the topical delivery of NSAIDs. Among these, piroxicam, ketoprofen, naproxen, tenoxicam may be mentioned [3–6].

Although the skin offers an effective barrier to the permeation of drugs, penetration enhancers reduce the barrier resistance of the stratum corneum and allow the drug to penetrate readily to viable tissues and enter the systemic circulation [7]. Fatty acids, alcohols and terpens can be used as enhancers [8, 9]. The degree of penetration depends significantly on the physicochemical properties of the active substance and the nature of the vehicle [10].

In addition, a recent study has shown that topical application of selective cyclooxygenase inhibitors suppresses UVB mediated cutaneous inflammation [11]. Therefore, topical application of celecoxib seems to have another important role in inhibiting UVB mediated inflammation as well as its systemic anti-inflammatory effects.

The object of this work was to study the influence of different topical formulations and of menthol and oleic acid as penetration enhancers on the release of celecoxib and its percutaneous penetration through human skin *in vitro*. It has been reported that *in vitro* models can be used as simple test models for new active agents with good correlation with *in vivo* results. They can be useful as basic test systems for comparing vehicles [12]. Oleic acid [13] and menthol [14] have been reported as being used as effective penetration enhancers for NSAIDs. On the basis of these reports, they were also used in our study.

2. Investigations, results and discussion

Solubility of celecoxib in the receptor phase was found to be 15.444 ± 0.209 mg/ml.

As can be seen from Table 1, when comparing different vehicles, it was found that HPMC gel as a donor phase gave the highest release, 932.317 mcg/cm^2, followed by 361.360 and 140.706 mcg/cm^2 for o/w emulsion and oleagenous cream respectively. Similarly in Table 2, the release rate of celecoxib from gel was found to be 182.827 mcg/cm^2/h, compared with the rates of 72.270 mcg/cm^2/h and 28.142 mcg/cm^2/h determined for o/w emulsion and oleagenous cream. In respect of release rates, statistically significantly different vehicle effects were found ($p < 0.05$). According to these data, it could be concluded that the best vehicle for celecoxib was the

Table 1: Amount of celecoxib released (mean ± SD) when incorporated in different vehicles (n = 6)

Base formulation	Time (h)	Amount of celecoxib released	
		mcg/cm^2	SD (±)
Gel	1	166.219	8.55
	2	400.687	13.92
	3	659.028	18.41
	4	756.244	21.13
	5	932.317	28.64
o/w Emulsion	1	69.786	9.14
	2	95.741	12.35
	3	177.537	17.14
	4	258.487	18.92
	5	361.360	17.06
Oleagenous cream	1	67.992	4.13
	2	83.842	7.32
	3	106.979	8.51
	4	122.059	10.40
	5	140.706	9.18

Table 2: *In vitro* release rate of celecoxib (mean ± SD) from the main formulations (n = 6)

Formulations	Release rate (mcg/cm^2/h)
Gel	182.827 ± 11.55
o/w Emulsion	72.270 ± 5.41
Oleagenous cream	28.142 ± 1.84

gel formulation. Our studies have shown clearly (Table 2 and Fig. 1) that incorporation of celecoxib in gel formulations increased the rate of release by up to 2.5–6.5 times when compared with o/w emulsion and oleagenous cream, respectively.

The influence of incorporation of oleic acid (2% and 5% conc.) and menthol (1% and 2% conc.) chemical penetration enhancers was also analysed in respect of penetration of celecoxib. It was found that in all vehicles incorporation of 5% oleic acid enhanced the penetration rate and increased the total penetration amount more than the other enhancers. As can be seen from Table 3, the penetration rate of celecoxib was found to be 0.233 mcg/cm^2/h from gel containing 5% oleic acid, compared with rates of 0.206 mcg/cm^2/h, 0.196 mcg/cm^2/h and 0.153 mcg/cm^2/h

Table 3: Penetration rate (mean ± SD) of celecoxib through human skin from the formulations (n ± 5)

Formulations		Penetration rate (mcg/cm^2/h)
Gel	1	0.206 ± 0.05
	2	0.200 ± 0.04
	3	0.213 ± 0.03
	4	0.236 ± 0.03
	5	0.233 ± 0.04
o/w Emulsion	6	0.196 ± 0.02
	7	0.183 ± 0.03
	8	0.203 ± 0.03
	9	0.230 ± 0.05
	10	0.232 ± 0.04
Oleagenous cream	11	0.153 ± 0.02
	12	0.155 ± 0.02
	13	0.175 ± 0.03
	14	0.178 ± 0.02
	15	0.227 ± 0.03

found with gel, o/w emulsion and oleagenous cream without enhancers, respectively.

In the penetration experiments, addition of oleic acid at 5% conc. significantly increased penetration rates (Table 3) and total penetration of celecoxib through human skin for all formulations tested (Figs. 2–4). In particular, this effect was most evident in oleagenous cream and can be attributed to the skin permeability enhancing effects of fatty acids. In Table 3, it will be observed that the penetration rate of celecoxib from oleagenous cream with 5% oleic acid was 0.227 mcg/cm^2/h, with the rate was found to be 0.153 mcg/cm^2/h for oleagenous cream without an enhancer. Increased skin/vehicle partitioning of the drug causes the enhancement of permeation by ion-pair formation between drug and fatty acids, resulting in the increase of partitioning into the stratum corneum. Conversely, menthol was chosen as the other penetration enhancer. Menthol has been reported as being used as an enhancer but in this study, it was not found to have any effect on the penetration of celecoxib. In Table 3, formulations 2, 3, 7, 8, 12 and 13 containing menthol were found to show no significant difference in respect of penetration rate when compared with formulations without enhancers (formulations 1, 6 and 11). In some cases, it even decreased the penetration rate (formulations 1, 2 and 6, 7). This effect could be due to interaction of the highly lipophilic

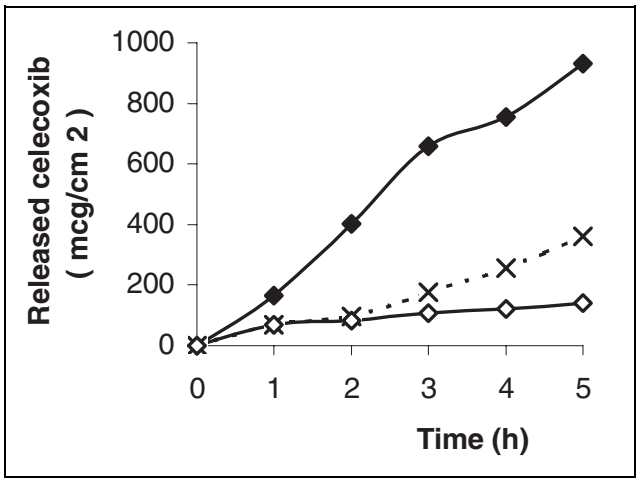

Fig. 1: Release profiles of celecoxib incorporated in different formulations. —◆— 1; ···×··· 6; —◇— 11

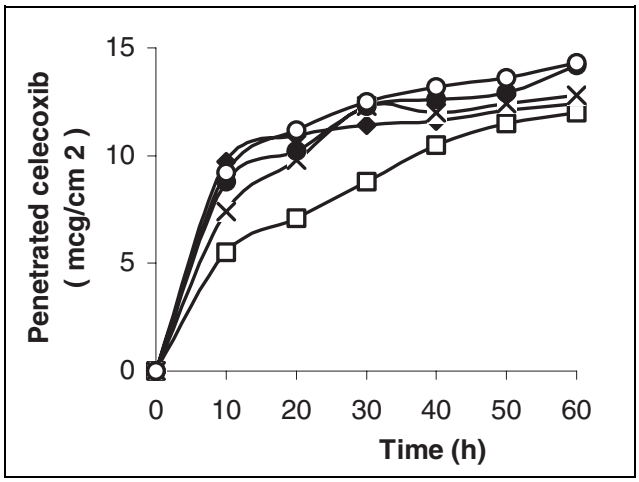

Fig. 2: Celecoxib penetration through human skin from HPMC gel in formulations 1–5. —◆— 1; —□— 2; —×— 3; —●— 4; —○— 5

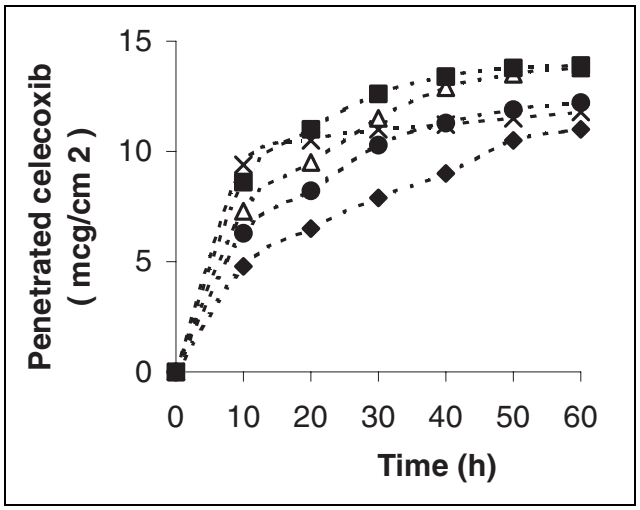

Fig. 3: Celecoxib penetration through human skin from o/w emulsion formulations 6–10. ···×··· 6; ···◆··· 7; ···●··· 8; ···△··· 9; ···■··· 10

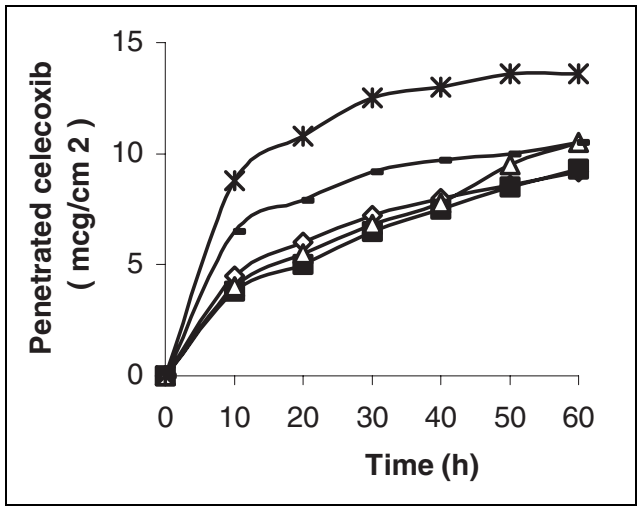

Fig. 4: Celecoxib penetration through human skin from oleagenous cream formulations 11–15. —◇— 11; —■— 12; —△— 13; —▬— 14; —✳—15

celecoxib with menthol. In some cases, penetration enhancers help lipophilic drugs to penetrate skin easily and in others, hydrophilic drugs. Many different factors influence the penetration rate and amount simultaneously.

Based on the results, it was concluded that a gel formulation including 5% oleic acid could be suggested as the most suitable carrier for celecoxib intended to be used topically. In addition, this system offers two main advantages. First, formulating celecoxib in a suitable vehicle will be useful in administering the drug topically to overcome gastrointestinal side effects, and secondly, since celecoxib has been found to suppress UVB mediated cutaneous inflammation, an acceptable topical carrier system could also be used in preparations for sun protection. Studies are still being carried out to determine the protective effects of celecoxib against UVB inflammations. It has also been shown that *in vitro* models can be used as simple test models for new active substances with good correlation with *in vivo* results.

The results indicate that the penetration of celecoxib from different topical formulations through excised human skin depends on the formulation and on the enhancer type and concentration. Penetration rate data indicated that was found to have the highest rate in a gel formulation, celecoxib followed by o/w emulsion and oleagenous cream.

3. Experimental

3.1. Materials and apparatus

Celecoxib (Searle), menthol (E. Merck), oleic acid (E. Merck), and all other reagents and chemicals were of analytical grade**.** Celecoxib was purchased and used in micronized form.

HPLC, UV detector, pump, injection port and 20 µl rheodyne (Hewlett-Packard series 1050), integrator (Hewlett Packard series 3396). Brownlee RP C_{18} column (25 × 0.4 cm, particle size 5 µm, part no: 0712-0016-OD-5A). UV spectrophotometer (Shimadzu UV-1601), Franz cells (Çalışkan Cam Teknik), cellulose acetate membrane (Sartorius).

3.2. Preparation of topical formulations

The compositions of the celecoxib gel, o/w emulsion and oleagenous cream used in this study are shown in Table 4. The enhancers were added to all these base formulations (Table 5).

3.3. Solubility studies

These studies were performed using to the method of USP XIX. 15 ml of a propylene glycol : water (60 : 40) mixture [15] as the receptor phase was placed in four 25-ml flasks. A quantity of celecoxib was placed in each flask which was greater than the quantity expected to dissolve in the receptor phase. The flasks were closed tightly and two of them were warmed to 30 °C. All the flasks were placed in a constant temperature water bath at 25 ± 1 °C. The apparatus was maintained under 200 rpm continuous agitation for 24 h and the dispersion was then filtered through blue ribbon filter paper (S & S 589[3]). A measured portion of the clear supernatant was removed using a pipet and diluted. The solubility of celecoxib in the receptor phase was determined spectrophotometrically at 272 nm.

Table 4: Composition (w/w%) of celecoxib topical base formulations

Constituents (%)	Base Formulations		
	Gel	o/w Emulsion	Oleagenous cream
Celecoxib	5	5	5
HPMC	3	–	–
Vaseline	–	–	95
Cetyl alcohol	–	23.75	–
White paraffine	–	23.75	–
Propylene glycol	–	11.4	–
Na lauryl sulphate	–	0.95	–
Distilled water	92	35.15	–

Table 5: Enhancers (w/w%) added to the base formulations

Formulation		Enhancer (%)	
		Menthol	Oleic acid
Gel	1*	–	–
	2	1	–
	3	2	–
	4	–	2
	5	–	5
o/w Emulsion	6*	–	–
	7	1	–
	8	2	–
	9	–	2
	10	–	5
Oleagenous cream	11*	–	–
	12	1	–
	13	2	–
	14	–	2
	15	–	5

* 1, 6 and 11 are the main formulations

3.4. In vitro release studies

The base formulations were used in this study (Table 4). A cellulose acetate membrane was used between the two halves of the diffusion cells. A propylene glycol : water (60 : 40) mixture was used as the receptor phase. Samples were taken from the receptor phase at given time intervals. The amount of celecoxib released was determined by UV spectroscopy at 272 nm.

3.5. Penetration studies through excised human skin

All the formulations were used in this study (Table 5). Franz diffusion cells with 1.76 cm^2 surface area and 10 ml receptor volume were used for the penetration studies. A propylene glycol : water (60 : 40) mixture was used as receptor phase and the whole assembly was placed in a water bath at 37 ± 1 °C.

Full-thickness human skin samples were obtained from the hospital following surgical operations. The underlying fatty tissue was removed by blunt dissection. 1 g of the formulations was applied on top of skin placed on the donor chamber of the diffusion cells. Three replicates were conducted for each experiment. Samples were taken over a 72 h period and celecoxib concentrations were determined by HPLC.

3.6. HPLC analysis

The celecoxib penetrated was analysed by HPLC. A mobile phase consisting of acetonitrile, water and isotonic potassium hydrogen phosphate buffer pH 7.2 (60 : 30 : 10) was used. Flow rate was 1 ml/min, injection volume was 20 µl and celecoxib was detected at 254 nm. The retention time of celecoxib was ~6.5 min.

3.7. Data treatment and statistics

The cumulative amount of celecoxib in the receptor compartment was plotted as a function of time. The steady state flux of celecoxib (µg/cm^2/h) was determined from the slope of the linear part of the plot using linear regression analysis ($r > 0.95$).

The amount of penetrant in the receptor compartment at the end of the experiment with different vehicles and penetration enhancer was compared using the ANOVA procedure. This procedure was carried out only after testing for normality. The flux in every cell was compared as a function of the vehicle and enhancer using the ANOVA procedure. The significance level was set as 5%.

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