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Effect of penetration enhancers on *in vitro* percutaneous penetration of nimesulide through rat skin

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The influence of several penetration enhancers alone and/or in various combinations on the percutaneous penetration of nimesulide (NM) from Carbopol® 934 based gel formulations was investigated. Skin permeation studies were performed using Franz-type diffusion cells and full-thickness abdominal rat skin. Various types of compounds such as ethanol, isopropyl alcohol, propylene glycol, Transcutol®, Tween® 80 and oleic acid were employed as penetration enhancers. The steady-state flux, the lag time and permeability coefficients of NM for each formulation were calculated. The results showed that the skin permeability of NM from gels tested was significantly increased ($P < 0.05$) by isopropyl alcohol (40%) and the combination of oleic acid (3%) with Transcutol® (30%) when compared with the control formulation. In conclusion, these substances could be considered as penetration enhancers for NM topical formulations.

1. Introduction

Transdermal therapy has become a useful drug delivery system in recent years. There are many reports on topical delivery of antiinflammatory drugs (NSAIDs) subjecting the possibility to avoid the gastrointestinal disturbances which might occur by oral administration (Wenkers and Lippold 2000; Gwak and Chun 2002). In addition, topical administration of NSAIDs offers the advantage to locally enhance drug delivery to affected tissues (Heyneman et al. 2000). However, one must bear in mind that the skin is a biological protective membrane against the entry of foreign compounds. Especially the Stratum corneum, the outermost layer of the skin, has physical barrier functions to most compounds, including drugs (Bach and Lippold 1998). Therefore, several studies have been performed with the objective of overcoming the low permeability of drugs through skin. One way to reduce this problem is to include permeation enhancers reducing the barrier characteristics of the Stratum corneum reversibly (Walters 1989).

Nimesulide (NM) is a NSAID reported to be a selective inhibitor of cyclo-oxygenase-2 (COX-2) (Davis and Brogden 1994). It has generally been administered orally and rectally for inflammatory diseases, fever and pain (Martindale 1996). Sengupta et al. (1998) demonstrated that topical administration of NM may also be a safe and effective alternative to oral and rectal routes. However only a few studies have been carried out to predict the percutaneous penetration of NM (Ceschel and Maffei 1999).

As a preliminary step, we investigated the influence of some penetration enhancers (ethanol, isopropyl alcohol, propylene glycol, Transcutol®, Tween® 80 and oleic acid) on the *in vitro* release of NM from gel formulations through a cellophane membrane (Güngör and Bergişadi 2003).

The objective of this paper was set to investigate the influence of these enhancers on *in vitro* percutaneous penetration of NM from gel formulations through abdominal rat skin. The effectiveness of these enhancers was evaluated *in vitro* using Franz-type diffusion cells.

2. Investigations, results and discussion

The *in vitro* penetration of NM through abdominal rat skin was investigated from gels based on Carbopol® 934 (Table 1). The pH value of the vehicle is a factor to be considered in the evaluation of drug penetration from gels through membranes or skin (Kushla and Zatz 1991). Thus gel formulations were adjusted to pH 6.0 ± 0.1.

The permeation profiles of NM from gel formulations (FC, F1–F7) through abdominal rat skin are shown in the Figure. The steady-state flux, the lag time, permeability coefficients and enhancement factors of NM for each formulation are summarized in Table 2.

Table 1: Composition of nimesulide gel formulations

Composition	Formula code							
	FC	F1	F2	F3	F4	F5	F6	F7
Nimesulide	1	1	1	1	1	1	1	1
Carbopol® 934	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl alcohol	–	–	30	–	–	40	–	–
Isopropyl alcohol	–	30	–	–	40	–	–	–
Propylene glycol	–	20	20	–	–	–	20	–
Transcutol®	–	–	–	40	–	–	–	30
Tween® 80	–	–	–	–	–	–	5	–
Oleic acid	–	–	–	–	–	–	–	3
Distilled water	98.5	48.5	48.5	58.5	58.5	58.5	73.5	65.5

Table 2: Permeation parameters of nimesulide from gels through rat skin[a]

Parameter	Formula code							
	FC	F1	F2	F3	F4	F5	F6	F7
J_{ss}[b] ($mcg \cdot cm^{-2} \cdot h^{-1}$)	7.93 ± 3.30	12.15 ± 4.56	8.12 ± 2.38	14.07 ± 5.09	16.88 ± 5.29[c]	10.19 ± 4.06	10.09 ± 5.17	15.79 ± 2.16[c]
t_L[b] (h)	2.98 ± 0.94	0.50 ± 0.48	0.76 ± 0.70	0.53 ± 0.18	0.33 ± 0.34	0.22 ± 0.24	2.19 ± 0.35	2.19 ± 0.50
Log K_p[b] ($cm \cdot h^{-1}$)	-3.14 ± 0.21	-2.93 ± 0.18	-3.10 ± 0.11	-2.91 ± 0.27	-2.78 ± 0.13	-3.01 ± 0.17	-3.05 ± 0.25	-2.80 ± 0.06
EF	1.00	1.53	1.02	1.77	2.13[c]	1.29	1.27	2.00[c]

[a] Data were expressed as the mean ± SD (n = 6)
[b] Skin permeation parameters: Nimesulide steady-state flux (J_{ss}), lag time (t_L) and permeability coefficient (K_p), enhancement factor (EF)
[c] Significantly higher than that of control ($P < 0.05$)

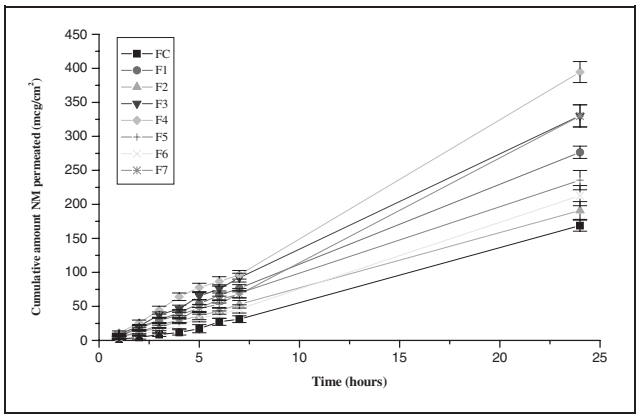

Fig: Permeation profiles of nimesulide from gels through rat skin (n = 6)

NM mean flux value of control gel formulation (FC) at steady-state was found as $7.93 \pm 3.30\ \mu g \cdot cm^{-2} \cdot h^{-1}$. Formulations F4 and F7 respectively containing isopropyl alcohol (40%) and the combination of oleic acid (3%) with Transcutol® (30%) showed significant increase in the NM flux when compared with the control formulation ($P < 0.05$). However, the other formulations (F1–F3, F5 and F6) showed insignificant increase in this respect ($P > 0.05$) (Table 2).

Various alcohols have been used as penetration enhancer in topical formulations. It has been reported that the enhancing effect of these alcohols on skin penetration occurs by increasing the permeability of Stratum corneum (Goto et al. 1993). In addition to altering the structure of the skin and modifying penetration rate, these enhancers act as a cosolvent and may affect solubility of the drug in the vehicle (Bach and Lippold 1998). Drug solubility influences the partition coefficient of the drug between the gel and the skin (Mura et al. 2000). According to our previous data, NM is sparingly soluble in the gel base and isopropyl alcohol added to the gel formulation increased drug solubility. Since NM was not completely dissolved in the vehicle, a suspension-type gel has been obtained (Güngör and Bergişadi 2003). Isopropyl alcohol, due to its solubilizing effect on NM, increases the drug concentration gradient in the gel and favours the passage of larger amounts of the drug into the Stratum corneum. For this reason, the enhancement effect of isopropyl alcohol on skin permeation of NM (F4) may be attributed to two mechanisms. First, isopropyl alcohol promotes drug release by increasing drug solubility (Güngör and Bergişadi 2003). Secondly, this penetration enhancer may interact with the polar groups of lipid components of the Stratum corneum (Takahashi et al. 1991).

Isopropyl alcohol significantly decreased the lag time (0.33 ± 0.34 h) of NM (F4) in comparison to control (2.98 ± 0.94 h) ($P < 0.001$), which would be an advantage for the rapid onset of the therapeutic effect of the drug.

In formulation F7, oleic acid and Transcutol® also led the increase in flux of NM when compared with control ($P < 0.05$) (Table 2). The enhancing effect of oleic acid could be attributed to the interaction between oleic acid and Stratum corneum lipids, increasing the fluidity of skin lipids and consequently drug flux (Barry 1987; Francoeur et al. 1990). Furthermore, the combination of oleic acid with Transcutol® is associated with the increase of drug mobility in the lipid regions of the skin and the increase of the drug solubility within those regions (Transcutol Product Profile 1995). The combination of oleic acid with cosolvents like Transcutol® has also been observed by several researchers (Barry 1987; Transcutol Product Profile, 1995; Larrucea et al. 2001). The increase in drug flux may be due to cosolvent enhanced intracellular drug mobility by solvating α-keratine in corneocyte and allowing oleic acid to act on the lipid barrier (Barry, 1987; Larrucea et. al. 2001).

Formulation F7 exhibited a longer lag time (2.19 ± 0.50 h) for NM showing no significant decrease in comparison to control ($P > 0.05$). This effect indicates that time is needed for oleic acid to penetrate through the skin and to interact with skin lipids (Santoyo et al. 1995).

In conclusion, the studies showed that isopropyl alcohol and the combination of oleic acid with Transcutol® could be considered as penetration enhancers for NM topical formulations.

3. Experimental

3.1. Materials

Nimesulide was supplied by Pfizer, Turkey. Transcutol® (diethylene glycol monoethyl ether) was a gift from Gattefossé, France. Carbopol® 934 was supplied by Goodrich Co., USA. Oleic acid, Tween® 80 and propylene glycol were purchased from Merck, Germany. Acetonitrile was purchased from J. T. Baker, Holland. All other chemicals were of analytical grade.

3.2. Preparation of the gels

The codes and composition of gel formulations used in this study are shown in Table 1. Carbopol® 934 was dispersed in distilled water. NM was added to the penetration enhancer(s) mentioned in Table 1. The latter was then incorporated into the carbomer dispersion. The gelation was achieved by the addition of triethanolamine. The control formulation (FC) containing no enhancer was also prepared using the same procedure. The final pH of all the formulations was adjusted to 6.0 ± 0.1 with triethanolamine. All the gels were stored at room temperature for 24 h under air tight conditions prior to use.

3.3. Permeation studies

The permeation studies were carried out using Franz-type diffusion cells with an effective diffusion area of 3.14 cm^2. The receptor compartment had a volume of 33.2 ml and was kept at 37 ± 0.5 °C using a water bath, circulator and jacket surrounding the cells. The receptor fluid was selected as 0.2 M phosphate buffer (pH 7.4) containing 25% (v/v) ethanol to maintain sink conditions (Moser et al. 2001) and stirred continuously with a Teflon-coated magnetic stirrer at 600 rpm. Full-thickness abdominal skin was excised from Wistar female rats (200–250 g) and hair of the rats were removed. Skins were allowed to hydrate with isotonic saline solution for 1 h before being mounted on the cells; dermal side was in contact with the

receptor compartment. 1 g of prepared gels were placed on the skin. After application, at scheduled time intervals 1 ml samples were taken from the receiver compartment and immediately replaced with the same volume of fresh receptor fluid. The amount of NM in the samples was determined by HPLC as described below. Permeation experiments were carried out until 24 h. Each experiment was run with three different rats in duplicate.

3.4. HPLC analysis

The content of NM in the receptor phase was analyzed by a HPLC system (Hewlett Packard 1100, Agillent series) consisting of a quartnery pump (Model G 1311A) and an autosampler (Model ALSG 1329A) using UV detection. The chromatographic resolutions were obtained with a C_{18} reverse-phase column (Luna, 5 μm, 15 cm × 4.6 mm). The mobile phase was 0.04 M potassium dihydrogene phosphate/acetonitrile/triethylamine (600 : 400 : 25; pH 3.0) and the flow rate was 1 ml/min. The UV detector (Model Colcom G1316A) was set at 225 nm. The quantitation limit of NM was 130 ng/ml. The linearity interval established was 0.15−120 μg/ml ($r^2 > 0.999$). Each experiment was carried out in triplicate. Calibrations were done by the external standard method.

3.5. Data treatment

The cumulative amount of drug penetrating through the abdominal rat skin was plotted against time. As described by Barry (1983) the steady-state drug flux (J_{ss}) is defined by Eq. (1):

$$J_{ss} = \frac{dQ}{A \cdot dt} \quad (1)$$

Where, dQ is the change in the quantity of drug passing through the skin expressed in μg, A is effective diffusion area (cm^2), dt is time (h). J_{ss} ($\mu g \cdot cm^{-2} \cdot h^{-1}$) was estimated from the slope of the straight line portion of the cumulative amount of drug permeated versus time and the lag time was determined from the x-intercept. The permeability coefficient (K_p) was calculated from the flux (J_{ss}) and initial concentration of NM in the donor compartment (C_v) using Eq. (2) (Roy 1997):

$$K_p = \frac{J_{ss}}{C_v} \quad (2)$$

The effectiveness of penetration enhancers (enhancement factor, E.F.) was determined as the ratio of drug flux at steady-state with enhancer to that of control (without enhancer) (Eq. 3).

$$EF = \frac{\text{NM flux at steady - state with enhancer}}{\text{NM flux at steady - state without enhancer}} \quad (3)$$

3.6. Statistical analysis

The values of permeation parameters obtained in each experiment were subjected to statistical analysis using the computer programme PC-Instat for a one-way analysis of variance followed by Student-Newman-Keuls multiple comparisons test. The chosen level of significance was $P < 0.05$.

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