Effect of Terpenes as Penetration Enhancers on Percutaneous Penetration of Tiaprofenic Acid Through Pig Skin

Tiyaprofenik Asidin Domuz Derisinden Geçişine Penetrasyon Artırıcılarının Etkisinin İncelenmesi

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Abstract

Tiaprofenic acid (TA) a nonsteroidal antiinflammatory agent that lipophilic character and widely used in rheumatoid arthritis via oral route. TA has various side-effects like other prostaglandine inhibitors in gastrointestinal system and these effects limit its use e.g., active stomach ulcer patients. Hence, local application of the drug as important as systemic application such cases mentioned above. The influence of different type terpenes in various combinations on percutaneous penetration of TA from Carbopol® 940 based gel formulations was investigated using Franz-type diffusion cells and pig skin. Four types of terpenes such as nerolidol, d-limonene, eucalyptol and menthol were employed as penetration enhancers and the steady-state flux, the lag time and permeability coefficients of TA for each formulation were calculated. The results of permeation studies showed that the rank order of enhancement ratio (ER_{flux}) for TA was d-limonene (5.89) > eucalyptol (4.73) > menthol (3.69) > nerolidol (1.43). Amongst the used terpenes in the gel formulations, d-limonene and eucalyptol were found to be an effective penetration enhancer for TA. However, further *in vivo* penetration studies should be performed to show the validity of the results of *ex vivo* penetration studies and to determine the therapeutic level of the drug.

Keywords: Tiaprofenic acid, terpenes, percutaneous penetration, pig skin, Carbopol® 940.

Introduction

The transdermal route offers several advantages over other routes for the delivery of drugs with systemic effect. It is easy and non-invasive route of application, furthermore no hospitalization is required. Additionally, the transdermal route as compared with the oral route

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reduces drug degradation at the site of administration due to its lower metabolic activity (Roy 1997). Once the drug is absorbed, the hepatic circulation is bypassed, thus avoiding another major site of potential drug metabolism e.g., intestinal metabolism (Barry 1983, Wester and Maibach 1992).

Despite the important advantages of transdermal drug delivery, this route of administration presents unique challenges. The greatest obstacle is *stratum corneum* (SC), the uppermost layer of the skin that provides the rate limiting step for drug transport. Therefore, several studies have been performed with the objective of overcoming the low permeability of drugs through skin. One of the way to reduce this problem is to include permeation enhancers reducing the barrier characteristics of *SC* reversibly (Marjukka et al. 1999) The use of penetration enhancer is valuable and important for achieving therapeutic plasma levels for many drugs in target tissue (Krishnaiah et al. 2006).

Terpenes, naturally occuring volatile oils, appear to be promising candidates for clinically acceptable enhancers (Williams and Barry 1991). Terpenes are generally considered as less toxic and have less irritant effects compared to surfactants and other skin penetration enhancers, and some terpenes have been characterized as Generally Recognized As Safe (GRAS) by the US FDA (Williams and Barry 1991, FDA 2006). They have high percutaneous enhancement ability, reversible effect on the lipids of *SC*, minimal percutaneous irritancy at low concentrations (1-5%) (Opdyke 1979, Okabe et al. 1990 and Obata et al. 1991). Moreover, a variety of terpenes have been shown to increase percutaneous absorption of both hydrophilic and lipophilic drugs (Zhao and Singh 1999, Gao and Singh 1998).

Tiaprofenic acid (TA) is a potent analgesic and nonsteroidal antiinflammatory drug (NSAID) and like other NSAIDs, the oral administration of conventional dosage forms of TA causes systemic side effects and gastric irritation (Martindale 2002). Transdermal formulation of TA has not been commercially available. In the literature, *in vitro* release of TA from different topical formulations (Carbopol® gel, chitosan gel, two types of emulsion based ointment formulations (o/w and w/o) and hydrophilic petrolatum USP) for examining the effect of vehicle composition was compared (Özsoy et al. 2004). The release characteristics of the formulations were evaluated using a standard cellophane membrane and Franz-type diffusion cells. The results indicated that Carbopol® 940 gel base exhibited significantly higher drug release than other vehicles.

The objective of this study was investigated the influence of some terpenes as a penetration enhancers (nerolidol, d-limonene, eucalyptol and menthol) on the *in vitro* percutaneous penetration through pig skin of TA from Carbopol® gel formulations.

Materials and methods

Materials

Tiaprofenic acid (TA) was kindly supplied by Aventis Pharma, Turkey. Carbopol® 940 was supplied by Goodrich Co., USA. Nerolidol and menthol were purchased from Merck. d-Limonene was supplied by Aldrich, USA. Eucalpytol was purchased from Fluka (Switzerland). All other chemicals were of analytical grade and supplied from Merck (Germany) unless stated otherwise.

Preparation of the gels

The codes and composition of gel formulations used in this study are shown in Table 1. The gel base (Carbopol® 940) was selected according to previous study (Özsoy et al. 2004) Briefly, Carbopol® 940 was dispersed in distilled water. TA was added to the penetration enhancer(s) mentioned in the Table 1. The latter was then incorporated into the carbomer dispersion. The gelation was achieved by the

addition of triethanolamine and the final pH of all the formulations was adjusted to 6.5±0.1 with triethanolamine as well. All the gels were stored at room temperature for 24 h under air tight conditions prior to use. Synergistic effects with ethanol were investigated by Obata *et al.* (1991) for some terpenes. Kobayashi and coworkers (1993) reported that an aqueous vehicle containing menthol and ethanol showed a marked enhancement effect not only on water-soluble drugs, but also on lipophilic drugs. In this study, it has been used as 20% (w/w).

Table 1. Composition of tiaprofenic acid gel formulations (w7w;%)

Composition	Formulation Code				
	F1	F2	F3	F4	F5*
Tiaprofenic acid	2	2	2	2	2
Carbopol® 940	1	1	1	1	1
Ethyl alcohol	20	20	20	20	20
d-Limonene	5	-	-	-	-
Nerolidol	-	5	-	-	-
Eucalyptol	-	-	5	-	-
Menthol	-	-	-	5	-
Distilled water q.s	100	100	100	100	100

Viscosity measurements

A Brookfield RVT viscosimeter (USA) was used to measure the viscosities (as cP) of gels prepared. A spindle (No. 3) was rotated at 30 rpm. Samples of the gels were measured at 25±1.0 °C.

Preparation of pig skin

As known, skin from the pig generally approximates the permeability of human skin (Dick and Scott 1999, Bhatia and Singh 1996). Thus, the percutaneous absorption of TA through pig skin can well be used to predict the percutaneous absorption in humans. Six mature pigs weighing 60-80 kg were used and anesthetized with sevofluoran under control of anesthesia specialist. Firstly, pigs were put the operating table, their hair was removed with scissors and 600 µm dorsal skin was carefully removed with adjustable hand dermatom (Bahadir Co., Samsun-Turkey). The skin samples were washed with water, wrapped aluminium foil and kept in -20°C deep freeze until used in the diffusion studies. After skin had been dermatomed conveniently, pigs were operated by hepatopancretobiliary surgeons for another study. This procedure has been carried out under approval of Istanbul University-Cerrahpasa Faculty of Medicine, Local Ethics Committee of Experimental Animals. The study was approved Summer-2006.

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 with circulator and jacket surrounding the cells. The receptor fluid was selected as 0.2M phosphate buffer (pH 7.4) and stirred continuously with a Teflon-coated magnetic stirrer at 600 rpm. The skins samples were allowed to hydrate with isotonic saline solution for 30 min before being mounted on the cells; dermal side was in contact with the receptor compartment. 1.0 g of prepared gel was placed on the skin. Permeation experiments were carried out until 24 h. After application, at scheduled time intervals (3, 4, 5, 6, 8 and 24 h) 250 µl samples were taken from the receiver compartment and immediately replaced with the same volume of fresh receptor fluid. The amount of TA in the samples was determined by high performance liquid chromatography (HPLC) method described below. The receptor fluid was injected to the HPLC directly in 20 µl volume with using microinsert vials.

HPLC analysis of TA was performed with slight modification described by Delbeke *et al.* (1997). HPLC system was the compact 2695 separation module Waters (USA) consisted of isocratic/gradient pump, autosampler, column heater, sample cooler, Waters 2487 double-beam UV/VIS detector and the Empower chromatography manager. Phenomenex RP C_{18} column (Varian, USA) 4.6x150 mm, 5 μm particle size was employed as stationary phase, with universal guard column holder and its C_{18} cartridges (Varian, USA). The mobile phase consisted of acetonitrile and water-glacial acetic acid [99:1, v/v], (40:60, v/v) prepared daily, filtered (0.45 μm membrane filter) and sonicated before use and was delivered at a flow rate of 0.8 ml/min. Column temperature 40°C and the detector was set at 310 nm. The retention time of TA was determined 7.9 min. Before commencing experiments the method was validated according to the standard procedures (Shah et al. 1991). These are linearity, intra- and inter-day precision, accuracy and specificity. Linearity of the calibration curves was shown in two concentration range. Peak area to concentration ratio over the concentration range was plotted. The slope, the intercept of the regression line and the coefficient of correlation were calculated. The linearity was shown in range of between 0.05 – 1.0 $\mu g.ml^{-1}$ and, 2-20 $\mu g.ml^{-1}$. Injection repeatability was determined in two concentrations and ten injections were carried out. Calculated relative standart deviations (RSD) were 0.56, 0.13 and 0.67 for 1, 5 and 10 $\mu g.ml^{-1}$, respectively. Precision is expressed as RSD and inter-day and intra-day reproducibility studies were performed on four different concentrations (n=6). Intra-day reproducibilities (RSD) were 1.91, 4.47, 3.07 and 3.81% for 10, 5, 1 and 0.5 $\mu g.ml^{-1}$, respectively. Inter-day reproducibilities (RSD) were detected 2.96, 5.39, 5.61 and 3.66 % for 10, 5, 1 and 0.5 $\mu g.ml^{-1}$, respectively. Accuracy represents the mean deviation from the nominal content of the sample (%). It is presented as percentage bias, and performed on four different concentrations (n=6). Mean deviation was calculated as 1.29, 0.80, 1.63 and -7.40 for concentration of 10, 5, 1 and 0.5 $\mu g.ml^{-1}$, respectively. The limit of quantification (LOQ) for TA was 0.05 $\mu g.ml^{-1}$. Limit of detection (LOD), the lowest concentration of analyte that the analytical process can reliably differentiate from the background level, at signal-to noise ratio of 3. The LOD value was calculated as 0.01 $\mu g.ml^{-1}$. Furthermore, no interfering peaks were detected during the analysis.

Data treatment

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

$$J_{ss} = \frac{dQ}{A.dt} \qquad (Eq.\ 1)$$

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} ($\mu g.cm^{-2}.h^{-1}$) was estimated from the slope of the straight line portion of (1-7 hours) the cumulative amount of drug permeated versus time enhancement ratios of flux (ER_{flux}) were calculated using Eq. (2) (Büyüktimkin et al. 1997).:

$$ER_{flux} = \frac{\text{Drug flux with percutaneous enhancer}}{\text{Drug flux without percutaneous enhancer}} \qquad (Eq.\ 2)$$

The permeability coefficient (K_p) was calculated from the flux (J_{ss}) and initial concentration of TA in the donor compartment (C_v) using Eq. (3) [20].

$$K_p = \frac{J_{ss}}{C_v} \qquad (Eq.\ 3)$$

The values reported are mean rates from a minimum of three replicates.

The values of permeation parameters obtained each experiment was subjected to statistical analysis using a 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 $p < 0.05$.

Results and Discussion

The viscosity values of all formulations are measured as > 2050 cP and found no significant difference ($p > 0.05$) between all formulations.

In our study, the enhancing effect of several terpenes, namely, d-limonene, nerolidol, eucalpytol and menthol, was investigated on *in vitro* permeation of TA through excised pig skin from Carbopol® gels. The permeation profiles of TA from gel formulations containing various terpenes through excised pig skin during 24 h are shown in Fig. 1.

Figure 1. Permeation profiles of tiaprofenic acid from gels through pig skin
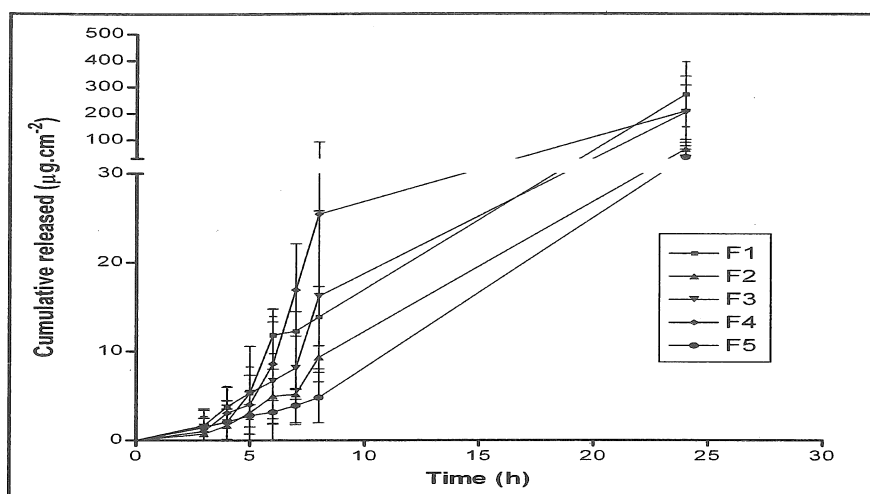
The steady-state flux (J_{ss}), permeability coefficient (K_p), lag time (t_L) and cumulative amount of TA after 24 h (Q_{24}) of TA for each formulation are summarized in Table 2.

Table 2. Permeation parameters of tiaprofenic acid from gels through pig skin[a]

Formulation Code	Parameters			
	J_{ss}[b] ($\mu g\ cm^{-2}h^{-1}$)	t_L[b] (h)	Log k_p[b] ($cm\ h^{-1}$)	Q_{24}[b] ($\mu g\ cm^{-2}$)
F1	13.95±6.96[c]	4.55±0.21	0.697±0.35[c]	272.48[c]
F2	3.38±1.32	4.18±1.28	0.169±0.07	66.54
F3	11.20±5.67[c]	5.78±1.78	0.560±0.28[c]	204.92[c]
F4	8.75±4.30[c]	4.80±0.51	0.417±0.23[c]	209.03[c]
F5	2.37±1.56	4.73±1.33	0.089±0.09	36.19

As seen in Table 2, the results of permeation studies through pig skin showed that steady-state flux values (J_{ss}) of TA from the formulations were 13.95, 11.20, 8.75 and 3.38 $\mu g\ cm^{-2}\ h^{-1}$ for F1, F3, F4 and F2, respectively. Most studies suggest that hydrophilic terpenes (alcohol, ketone, and oxide terpenes) are more effective in enhancing the permeation of hydrophilic

drugs, whereas hydrocarbon terpenes (d-limonene and cymene) are more active in promoting percutaneous permeation of lipophilic drugs (Moghimi 1997). d-Limonene, a hydrophobic terpene without a hydroxyl group (lipophilicity indicated by log P is 4.58±0.23) (El-Kattan 2001) was reported that d-limonene was very effective in enhancing the transport of lipophilic molecules such as indomethacin (Okabe 1989) ketoprofen (Okabe et al. 1990), butyl paraben (Koyama 1994), midazolam (Ota 2003) and estradiol (Monti et al. 2002) It is possible that d-limonene might increase the drug permeation by partitioning the drug into the SC lipids (Krishnaiah 2003) TA is a lipophilic character predicted log P of 2.42 calculated using ADC Software (Advanced Chemical Development Inc. Toronto, Canada, Version 3.5) (Hadgraft 2000) In this study, d-limonene was found as the best enhancer with more than 7-fold increase in the permeability coefficient of TA.

On the other hand, eucalpytol showed significant increase ($p<0.05$) in J_{ss} when compared with control formulation (Table 2). Log P value of eucalyptol is 2.82 (Cal 2006). It has been used as penetration enhancer for propranolol HCl (Amnuaikita 2005) and zidovudine (Narishetty 2005). There is no significant between J_{ss} values of d-limonene and eucalpytol (p>0.05). In addition, d-limonene and eucalyptol also provided the highest Q_{24} value (272.48 µg cm^{-2}, 204.92 µg cm^{-2}, respectively) (Table 2).

Menthol (log P= 3.20) (Kang 2007) has been used as an enhancer for transdermal delivery of variety of drugs including imipramine HCl (Jain 2002) caffeine, hydrocortisone, triamcinolone (Godwin 1999) propranolol HCl (Amnuaikita 2005).As can be understand from this study menthol is commonly increased hydrophilic drug penetration furthermore, it was reported that menthol did not increase of etodolac, a lipophilic anti-inflammatory agent, permeation in rat skin (Tas 2007). Because of this feature nerolidol has shown higher flux value of TA (8.75±4.30 µg cm^{-2}h^{-1}) after, that of d-limonene and eucalyptol.

As expected, TA permeation from containing nerolidol was increased from pig skin significantly (p<0.05) higher than control. Because, nerolidol, a sesquiterpene alcohol (lipophilicity as donated by log P is 5.36±0.38) (El-Kattan 2000) is found to be good candidate for the enhancement of hydrophilic drugs rather than lipophilic drugs (El-Kattan 2001,Güngör et al. 2008). Nerolidol provided the best enhancement activity for hydrophilic drugs such as diclofenac sodium (Nokhodchi 2007) and hydrocortisone (El-Kattan 2001), 5-Fluorouracil (5-FU) (Cornwell 1994) and nicardipine HCl (El-Kattan 2001) permeation. In addition, it was informed that the penetration of terpenes into SC is greater if their log P value is close to 3 (Cal 2006). Nerolidol has highest log P value (log P= 5.32) between other terpenes using in this study and consequently was shown lowest drug permeation when compared with d-limonene, eucalyptol and menthol. This finding is reported in the literature (Cal 2006).

In this study, nerolidol which has the highest log P value (log P= 5.32) was ensured the highest permeation from pig skin with 4.18 h lag time. As for eucalyptol has got lowest log P value (log P =2.82) and caused by highest lag time (t_L= 5.78 h). As known, a correlation existed between the log P of terpenes and the lag time. When the log P increased, a linear decrease in lag time was observed. Graphic which is drawn with using determination of lag time and log P value of terpenes using in this study was shown that highest correlation (r^2=0.777) (Fig. 2). A similar report existed in elsewhere (Tas 2007).

Figure 2. The relationship between the terpene enhancer log P and tiaprofenic acid lag time using pig skin (n=6) (r2=0.777, p<0.01)
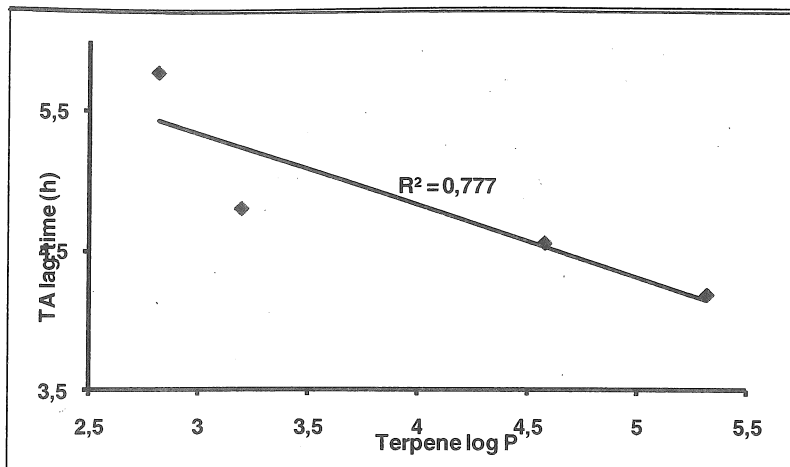
The results of in vitro permeation studies showed that the rank order of enhancement ratio (ER_{flux}) for TA was d-limonene (5.89±2.94) > eucalyptol (4.73±2.39) > menthol (3.52±1.97) > nerolidol (1.54±0.53) (Fig. 3). Amongst the employed terpenes in the gel formulations, d-limonene and eucalyptol were found to be an effective penetration enhancer for TA.

Figure 3. The comparison of enhancement ratio of tiaprofenic acid flux (ER_{flux}) of terpenes employed. ER_{flux} value of d-limonene and eucalyptol are statistically significant as compare to nerolidol (p<0.05).
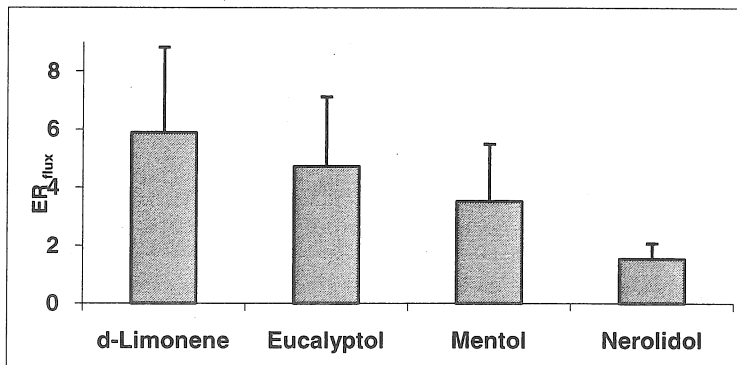
In conclusion, data obtained from *in vitro* permeation studies indicate that permeation studies through pig skin of TA suggested that d-limonene and eucalyptol are the most effective enhancers among terpenes using in the Carbopol® gel formulation. However, further *in vivo* penetration studies should be performed to show the validity of the results of *ex vivo* penetration studies and to determine the therapeutic level of the drug.

253

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