EFFECT OF THE VARIOUS PENETRATION ENHANCERS ON THE IN VITRO SKIN PERMEATION OF MELOXICAM THROUGH WHOLE RAT SKIN

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ABSTRACT

Purpose: The main aim of this research was to investigate the effect of various penetration enhancers on the in vitro skin permeability of meloxicam. Methods: meloxicam permeability parameters through whole rat skin were studied with and without chemical penetration enhancers such as Eucalyptus oil, oleic acid and Transcutol p. Results: The skin showed barrier for meloxicam permeability through full skin and that diffusion into the skin was the rate-limiting step for drug flux. Transcutol P, eucalyptus oil and oleic acid were the most effective enhancers as they increased flux 20.7, 16.54 and 13.54 times and diffusion coefficient 17.18, 12.94 and 13.05 times in comparison with hydrated skin, respectively. Chemical penetration enhancers are materials that disrupt the lipid and lipid-protein complex structure of the skin. Two endothermic transitions were obtained at around 67°C (Tm1) and 112°C (Tm2) were obtained in thermogram of hydrated whole rat skin. Tm1 and Tm2 transitions appeared to be due to melting of lipids and irreversible denaturation of intracellular keratin or melting of lipid – protein( keratin) complex ,respectively. Tm1, ΔH1 and ΔH2 were shifted by all the penetration enhancers to lower amounts in comparison with hydrated skin. Conclusion: All the chemical penetration enhancers showed markedly increase the permeation of meloxicam through whole rat skin in comparison with control.

KEYWORDS:  meloxicam, percutaneous absorption, penetration enhancers, differential scanning calorimeter.
INTRODUCTION

The transdermal route has been recognized as one of the highly potential routes of systemic drug delivery as skin is an important site of drug application for both local and systemic effects. Permeation of drugs through the skin is the basis of transdermal delivery. The use of a topical drug for dermal and transdermal drug delivery depends on the ability of the drug to penetrate via skin in sufficient quantities to achieve the desired effect. Transdermal route provides many advantages among them, avoidance of first pass metabolism of drug, controlled drug delivery, continuous drug delivery, reduction of dosing frequency, patient compliance enhancement, facilitation of drug localization at target site, reduction in toxicity of drugs, avoidance of the risks of injections.\[1\] Permeation of drugs through the skin is the basis of transdermal delivery. The two essential steps in skin permeation of drugs are partitioning and diffusion through the stratum corneum and then viable epidermis, passage into the dermis and finally, systemic absorption. The stratum corneum provides the greatest resistance to penetration, and it is the rate-limiting step in percutaneous absorption.\[2\] The stratum corneum poses a powerful challenge to drug delivery systems. Several possible strategies have been used for improving the drug permeation into lower skin layer and deeper tissues. Penetration enhancement technology is a challenging development that would increase the number of drugs available for transdermal administration. Penetration enhancers are the substances that facilitate the absorption of penetrant through the skin by temporarily diminishing the impermeability of the skin. Chemical penetration enhancers have been designed to facilitate delivery of high drug concentrations across the skin into systemic circulation or deeper tissues.\[3\] There are a variety of mechanisms for penetration enhancement by these substances.\[4\] Meloxicam is a potent NSAIDs that inhibits COX 2 and has analgesic and anti-inflammatory activity, widely used in the treatment of rheumatoid arthritis, osteoarthritis and other joint disease.

The oral efficiency dose (7.5-15 mg/day) of meloxicam is the lowest in the nonsteroidal anti-inflammatory drugs.\[5\] Although meloxicam Preferentially inhibits COX-2 (cyclooxygenase-2) over COX-1 (which is responsible for physiological processes in the stomach and kidney) its oral administration can produces gastrointestinal side effect.\[6,7\] The increased incidence of inflammatory joint diseases has necessitated the need to search for new topical dosage form of meloxicam. Meloxicam also possesses appropriate physiochemical properties for potential transdermal delivery such as low molecular weight(354.1), low polarity, low melting point and low daily therapeutic dose.\[8-10\]
The aim of this research work, therefore, is to develop a topical meloxicam delivery system and evaluate the effect of various penetration enhancers on the in vitro skin permeability characteristics of meloxicam.

MATERIALS AND METHODS

Materials
Meloxicam was purchased from Iran Hormone pharmaceutical company (Tehran, Iran). Eucalyptus oil, containing 70% 1,8-cineole, was obtained from Barij Essence Iranian Company in Kashan (Iran). Diethylene glycol monoethyl ether (Transcutol P) was obtained as gift samples (Gattefosse, Saint-Priest, France). Oleic acid, potassium phosphate monobasic and dibasic were purchased from merck company. Water was deionized and filtered in-house was used.

Animal experiments
Male Wistar rats (weighing 200-250 g) and aged 8-10 weeks were purchased from Animals Laboratory, Jundishapour University of Medical Sciences, Ahvaz, Iran. The hair on the abdominal skin was carefully removed with an electric clipper, taking care not to damage the skin. The rats were anaesthesized with ether prior to sacrificing them. After sacrificing under ether anesthesia, the abdominal skin hair was carefully removed with an electric clipper and without damaging. The skin was excised and any extraneous subcutaneous fat was cleaned from the dermal surface using cooled pure acetone solution with 4°C. Whole skin thickness was measured using a digital micrometer (AACO Company, France). The animals were treated according to the principles for the care and use of laboratory animals and approval for the studies was given by the Ethical Committee of the Ahvaz Jundishapour University of Medical Sciences.[11]

Meloxicam assay
The amount determination of meloxicam was carried out by UV spectroscopy method (BioWaveII, WPA) at \( \lambda_{\text{max}} = 362 \) nm.

In vitro skin permeation study
In-vitro permeation studies were carried out using vertical glass diffusion cells fabricated in house with an effective diffusion area of approximately 3.46 cm². The volume of the receptor compartment was 25 ml. Whole skin samples, hydrated prior to use, were mounted between the donor and receptor compartments of the cells with the epiderm facing the donor medium.
Meloxicam (0.3 %w/v), dissolved in the distilled water, was in the donor compartment and the receptor cell was filled with phosphate buffer solution (pH 7). The diffusion cell was placed and clamped in a water bath 37 ± 0.05 °C placed on a magnetic stirrer with a heater. The receptor medium was stirred with a small magnetic bead at 300 rpm. At predetermined time intervals (0.5, 1, 2, 3,4,5……,72 h), a 2 ml of the receptor medium was withdrawn and immediately replaced with an equal volume of fresh phosphate buffer solution to maintain sink condition. The samples filtered and the permeated amount of meloxicam was determined by UV spectroscopy method at 362 nm.[2]

**Differential scanning calorimeter (DSC)**

The changes in structure of whole skin induced by permeation enhancers were examined using a DSC (Mettler Toledo DSC1 system) equipped. The fully hydrated skin samples were first immersed in a chemical permeation enhancer for 2 h and the excess of the enhancer was blotted out before they were hermetically sealed to avoid evaporation of water. Approximately 6–10 mg of pretreated skin samples were placed in hermetically sealed aluminum pans. Simultaneously an empty hermetically sealed pan was used as a reference. Skin samples were exposed to heat ranging from 20 to 200 °C (scan rate: 5 °C/min). All experiments were at least in triplicate. In order to ensure accuracy and repeatability of data, DSC analyzer was calibrated and checked with indium standard.[12]

**Pretreatment of skin samples with chemical penetration enhancers**

For pretreatment of skin samples, fully hydrated samples were pretreated with putting 2 ml of a chemical enhancer on the surface of skin in the donor phase for 2 hours. The donor and receptor compartments were then washed with water and filled with aqueous solution of meloxicam and phosphate buffer (pH 7), respectively. The effect of chemical enhancers was evaluated for meloxicam permeation through whole skin samples. Transcutol p, oleic acid and eucalyptus oil (containing 70 % 1,8-cineole) were used as chemical penetration enhancers. Fully hydrated samples were used as controls. To minimize experimental errors arising from biological variability, each piece of skin was used as its own control.[13]

**Permeation Data analysis**

The cumulative amount of meloxicam permeated through unit area of the diffusion surface into the receptor was calculated and plotted as a function of time. Steady-state flux (mg/cm2/h) was calculated from the linear portion of the slope of the permeation curve.
Permeability coefficient \((K_p, \text{cm/h})\) of meloxicam through the skin was calculated as in Eq 1 (14):

\[ K_p = \frac{J_{ss}}{C_v} \]  

where \(J_{ss}\) is steady-state flux and \(C_v\) the initial concentration of meloxicam in the donor compartment.

Enhancement ratios \((ER)\) were calculated from permeation parameters after enhancer treatment divided by the same parameters before enhancer treatment (Eq 2).

\[ ER = \frac{\text{permeability parameter after treatment}}{\text{permeability parameter before treatment}} \]  

Statistical comparison was made using one-way ANOVA and \(p < 0.05\) was considered statistically significant. Correlation analysis was performed by least square linear regression method and correlation coefficient examined for significance by Student’s \(t\)-test. All statistical analyses were conducted using MINITAB software (version 16.0).

RESULTS AND DISCUSSION

Enhancing effect of enhancers on In vitro permeation of Meloxicam

Permeability parameters after skin pretreatment with penetration enhancers compared to control are displayed in table 1. The effect of penetration enhancers on meloxicam permeability (hydrated skin as control) is expressed in table 2 as \(ER_{flux}\) (ratio of drug flux after and before skin pretreatment with enhancer) and \(ER_D\) (drug diffusion coefficient after and before skin pretreatment with enhancer). The results indicate that all penetration enhancers increased meloxicam flux and diffusion coefficient significantly \((p<0.05)\). Transcutol p provided the best enhancing effect on meloxicam, followed by eucalyptus oil and oleic acid in that order. The enhancement effect is approximately 20.79 times for transcutol P, followed by eucalyptus oil (16.54 times) and oleic acid (13.54 times). All the penetration enhancers showed significant increase in diffusion coefficient \((p< 0.05)\), with transcutol showing the highest enhancement effect on diffusion coefficient (17.8 times) followed by oleic acid (13.05 times) and eucalyptus oil (12.94 times). \(ER_{flux}\) of all penetration enhancers were higher than their ERD, indicating that the penetration enhancers increased flux more than they did diffusion coefficient .The enhancing effect of all penetration enhancers on flux was same as diffusion coefficient. This observation indicates that the principal mode of action of Transcutol P enhancement activity is facilitation of drug partitioning and diffusion into skin. Transcutol is a powerful solubilizing agent and is miscible with polar and non-polar solvent. Eucalyptus oil consists of 75 % 1,8- cineole that
Cineole is a cyclic terpene. The mechanism of action of terpenes involves one or more of effects consist of: increasing diffusion coefficient, drug solubility (i.e., increasing the thermodynamic activity of the drug), partition coefficient, lipid extraction (i.e., disruption of lipid – protein domain). Oleic acid has shown to be effective for many drugs. It was revealed that oleic acid might increase the permeability via a mechanism involving perturbation of stratum corneum lipid bilayer and lacunae formation as penetration enhancing effect.

The two main steps in skin penetration are partitioning and diffusion through the stratum corneum, partitioning and diffusion to the viable epidermis, passage into the dermis and finally, systemic absorption or penetration into deeper tissues.

Meloxicam falls in Biopharmaceutical Classification System (BCS) class II, so that the low solubility of meloxicam in water suggests that the rate-limiting step for meloxicam penetration through whole rat skin is the diffusion through stratum corneum to vital epidermis. Meloxicam with lipophilic properties demonstrates good partitioning from aqueous solution into skin and so partitioning into stratum corneum isn’t rate limiting step. Therefore low permeation is due to diffusion through skin layers.

**Differential scanning calorimetry (DSC)**

Thermotropic behavior of skin treated with various chemical penetration enhancers determined by comparing for mean transition temperature (Tm) and their enthalpies (ΔH). The temperature transition and enthalpy amounts after enhancers treatment are shown in Table 3. In the Previous studies, Vaddi et al. reported three endothermic transition at 62, 79, and 95°C in the thermotropic behavior of rat skin, while Shakeel et al. observed 4 endothermic transitions at 34, 82, 105, and 114°C. Kaushik et al. studied the SC of human skin and observed three endothermic transition peaks at temperatures 59 - 63°C (Tm₁), 75 - 82°C (Tm₂) and 99.5 - 120°C (Tm₃). It has been shown that Tm₁ corresponded to lipid transformation from a lamellar to disordered state, Tm₂ is due to the melting of lipid – protein (keratin) complex. Tm₃ is known to occur during the irreversible denaturation of proteins in the SC.

The thermogram of hydrated whole rat skin is shown in Fig 1. In our study, Two endothermic transitions as obtained from the DSC thermogram of hydrated whole rat skin were observed around 67 (Tm₁) and 112°C (Tm₂). Tm₁ and Tm₂ transitions appeared to be due to melting of
lipids and irreversible denaturation of intracellular keratin or melting of lipid – protein (keratin) complex, respectively. The shift in Tm to lower temperatures can be interpreted as disruption of the lipid bilayer and the irreversible denaturation of proteins in the SC layer of skin, while decrease in ΔH is related to fluidization of lipid in lipid bilayer and lipid – protein complex.\[18-21\] Thermograms obtained from pretreatment of whole skin with all the penetration enhancers observed that Tm\(_1\) and ΔH\(_1\) were shifted to lower temperatures and enthalpy. Transcutol P, eucalyptus oil and oleic acid were provided the highest decreasing at T\(_{67}\) and ΔH\(_1\), respectively. These observations indicate that all penetration enhancers caused disruption of the lipid bilayer and increased lipid fluidity in intercellular region. On the other hand, all the penetration enhancers caused T\(_{112}\) shift to higher melting points and lower ΔH\(_2\), suggesting possible fluidization lipid – protein complex in the SC layer of skin.\[21\] However, we can propose that the lipid bilayer (T\(_{67}\)) response to the main barrier for meloxicam diffusion and diffusion through this lipid structure was rate-limiting for drug flux.

Table 1: Permeability parameters after pretreatment with penetration enhancers compared to control (mean ± standard deviation, n = 3)

<table>
<thead>
<tr>
<th>parameter</th>
<th>Flux (mg.cm(^{-2}.h(^{-1}))</th>
<th>D (cm(^2.h(^{-1}))</th>
<th>T(_{lag}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Water)</td>
<td>0.0036 ±0.0003</td>
<td>0.0021 ±0.0012</td>
<td>35.61± 2.53</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.0485 ±0.0015</td>
<td>0.0230 ±0.0016</td>
<td>2.36 ±0.17</td>
</tr>
<tr>
<td>Transcutol p</td>
<td>0.0746 ±0.0004</td>
<td>0.0302 ±0.0017</td>
<td>1.79 ±0.10</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>0.0592 ±0.003</td>
<td>0.0223±0.0001</td>
<td>2.42 ±0.01</td>
</tr>
</tbody>
</table>

Table 2: Effect of penetration enhancers on permeation parameters for meloxicam through whole skin rat (mean ± standard deviation, n= 3)

<table>
<thead>
<tr>
<th>penetration enhancer</th>
<th>ER(_{flux})</th>
<th>ER(_D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>13.54 ±1.47</td>
<td>13.05± 6.85</td>
</tr>
<tr>
<td>Transcutol</td>
<td>20.79 ±1.75</td>
<td>17.18 ± 9.24</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>16.54 ±2.10</td>
<td>12.94± 7.54</td>
</tr>
</tbody>
</table>

\(ER_{flux} = \) ratio of flux after and before treatment with enhancer; \(ER_D = \) ratio of diffusion coefficient after and before treatment with enhancer.
Table 3: Effect of penetration enhancer on the thermal properties of hydrated rat skin (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>Penetration Enhancer</th>
<th>Transition temperature (°C)</th>
<th>Transition enthalpy (mJ/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_m^1$</td>
<td>$T_m^2$</td>
</tr>
<tr>
<td>Control (Water)</td>
<td>67±2.1</td>
<td>112±6.6</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>51±2</td>
<td>135±2.3</td>
</tr>
<tr>
<td>Transcutol P</td>
<td>48±1.1</td>
<td>117±1.2</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>41±1.8</td>
<td>146±2.1</td>
</tr>
</tbody>
</table>

$T_m^1$ = mean transition temperature of lipids; SC $T_m^2$ = mean transition temperature of irreversible denaturation of intracellular stratum corneum keratin; $\Delta H_1$ = transition enthalpy of lipid phase SC $\Delta H_2$ = transition enthalpy of keratin phase SC

**Fig. 2**: DSC Thermogram of Hydrated whole skin rat

**CONCLUSION**

In the present study, it is clear that all the chemical penetration enhancers showed markedly increase the permeation of meloxicam through whole rat skin in comparison with control. Disruption of the lipid bilayer and fluidization of lipid in lipid bilayer and lipid – protein complex are main factors for higher ER$_{flux}$ and ER$_{D}$. The main barrier in meloxicam permeability is diffusion through skin layers, and therefore, to achieve a good percutaneous formulation with satisfactory dermal penetration, incorporation of transcutol P, eucalyptus oil and oleic acid would be of great advantage.
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REFERENCES


