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Original Article

An Investigation into the Effect of Various Penetration Enhancers on Percutaneous Absorption of Piroxicam

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Abstract

Achieving a desirable percutaneous absorption of drug molecule is a major concern in formulating dermatological products. The use of penetration enhancers could provide a successful mean for this purpose. The aim of this study was to evaluate the effect of incorporating a few common penetration enhancers (in different concentrations) into a 0.5% w/w piroxicam (model drug) gel formulation, on the permeability rate of drug through rat abdominal skin *in vitro*. For this purpose various concentrations of oleic acid (OA), urea (UR), lecithin (LEC) and isopropyl myristate (IPM) were used as the penetration enhancer. In order to investigate the effect of penetration enhancers used in this study on the permeability rate of piroxicam through sections of excised rat skin, Franz-type diffusion cells were employed. The receptor phase was constantly stirring 0.9% w/v sodium chloride solution at 32°C. At set intervals up to 8h, 5ml samples were removed from the receptor compartment and the amount of piroxicam permeated through the skin calculated by determining the UV absorbance of drug at 353 nm. Results show that among the penetration enhancers used, the use of OA at a concentration of 1.0% w/w had the greatest effect on the permeability rate of piroxicam, and produced the highest enhancement ratio among all the penetration enhancers examined. The other penetration enhancers used were found to have a far smaller effect on the permeability rate of piroxicam through rat skin. The enhancement ratio of the penetration enhancers used in the formulation of piroxicam gel were found to increase in the order of OA>> IPM > LEC > UR.

Keywords: Piroxicam gel; Percutaneous absorption; Skin permeability rate; Penetration enhancer; Enhancement ratio; Oleic acid; Isopropyl myristate.

Introduction

Drug delivery to or via skin has provided an effective route for local or systemic administration of therapeutically active agents. However, skin and in particular stratum corneum provide an efficient protective barrier for drug absorption. Stratum corneum (horny layer) has a thickness of about 15-20 μm, and is a layer of compressed, overlapping keratinized cells that form a flexible, tough and coherent membrane. This layer contains dead cells with keratin filaments in a matrix of proteins with lipids and water-soluble substances. The thickness and penetration properties of stratum corneum depend upon its hydration, which normally contains around 20% water (1, 2). Hence, one of the major challenges facing formulation scientists is to enhance and improve drug absorption through this rather elaborated barrier.

A useful approach for increasing percutaneous absorption of drugs is to employ penetration or permeation enhancers. These are agents that partition into, and interact with skin constituents to induce a temporary and reversible increase in skin permeability (3, 4).

Various materials have been mentioned in the literature as penetration enhancer. Some of these materials include alcohols and glycols such as ethanol, propanol, butanol and propyl-

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ene glycol; alkyl methyl sulfoxides such as dimethyl sulfoxide; pyrrolidones and their derivatives such as N-methyl-2-pyrrolidone; fatty acids and alcohols such as oleic acid, lauric acid, lauryl alcohol and stearic acid; various surfactants such as sodium lauryl sulfate and tween 20; lecithin; azone; urea and its derivatives; isopropyl myristate; and terpenes and terpenoids such as d-limonene and 1, 8-cineole (7-12).

A number of drugs have been investigated for their percutaneous absorption, among which is the non-steroidal anti-inflammatory drug (NSAID) piroxicam, used as the model drug in this study.

In a study by Vincent and co-workers (13) the *in vitro* release efficacy of ketoprofen and piroxicam across human skin was ranked higher than the other NSAIDs tested. In another study several terpenes (thymol, menthone and 1, 8-cineole) were found to increase the flux of piroxicam across skin membrane, when used as passive and iontophoretic skin pretreatment (14).

In a recent study surfactants possessing anionic and cationic head groups were found to be more potent than those possessing nonionic head groups in increasing skin conductivity in the presence of ultrasound. Furthermore, for surfactants possessing the same head group, those with 14-carbon tail length were found to be most effective in enhancing skin permeability (15).

Okuyama and co-workers have found that the ethylene oxide chain length of polyoxyethylene nonionic surfactants from 5 to 15, enhanced percutaneous absorption of piroxicam to a greater extent than that of the other chain lengths, when tested invivo in guinea pig (16).

Finally, the use of oleic and linoleic acid as penetration enhancer was found to be effective in enhancing the skin permeation of tenoxicam (17).

The aim of this study was to investigate the effect of various common penetration enhancers, when used in different concentrations, on the percutaneous absorption of piroxicam *in vitro*. Furthermore, the enhancement ratio of these penetration enhancers at different concentrations has been tabulated and contrasted.

Experimental

Materials

Oleic acid, urea, isopropyl myristate, propylene glycol, sodium chloride and sodium lauryl sulfate were all purchased from Merck Chemical Co., Germany. Lecithin (Epikuron 200) was obtained from Lucas Mayer Co., Germany. Reference standard piroxicam powder was a gift from Iran's FDA. Hydroxypropylmethyl cellulose (HPMC) was obtained from Colorcon Ltd., U.K.

Methods

Preparation of test gels

In this study various penetration enhancers were incorporated into a hydroalcoholic gel formulation, prepared based on previous studies and containing 0.5% w/w piroxicam as the active ingredient as well as HPMC and propylene glycol as inactive ingredients, and their effects on the permeability rate of piroxicam through rat skin were evaluated. The penetration enhancers used in this study included oleic acid (OA), urea (UR), lecithin (LEC) and isopropyl myristate (IPM). Various amounts of these penetration enhancers were incorporated into the gel in order to form a uniform formulation. It should also be mentioned that the apparent viscosity of gels containing penetration enhancers used was similar to that of the original piroxicam gel (with no penetration enhancer) and remained almost unchanged. The resulting gels were then used for skin permeation studies.

Skin permeation studies

For the purpose of this study skin from the abdominal area of young male N-marry rats weighing 250 ± 30 g was used. Rats were sacrificed by placing them in an ether saturated desiccator. Next, hairs present within the abdominal area of the animal were carefully cut as short as possible using scissors, without damaging or scratching the skin surface. In the next stage the skin was surgically removed, cleaned from muscle, fat or vasculature and used for experimentation.

Skins prepared were then individually placed in static Franz-type diffusion cells, designed in-house. Fifty milliliters of a 0.9% w/v sodium chloride solution was used as the receptor phase. Excised sections of rat skin were then carefully placed between the donor and receptor

compartments of the diffusion cell and firmly stuck in place using a liquid cyanoacrylate adhesive. Assurance was made that the skin is fully in contact with the receptor phase, leaving out any air bubbles. Next, the cell cap was placed on the donor compartment and the areas around the cell cap as well as the withdrawal port (tube) for removing the receptor phase (within the receptor compartment) were tightly closed using parafilm, to avoid contact with the exterior environment. Then, this set up was stored at 4°C for a period of 20-24 h in order to allow complete hydration of the skin. Following this period the receptor phase was completely removed and replaced with fresh 0.9% w/v sodium chloride solution. Next, 300 mg/cm^2 of piroxicam gel were uniformly placed in the donor phase, in contact with the excised section of rat skin. The overall area of skin covered was 6 cm^2. Finally, the cell cap was again placed over the donor compartment and the areas around the cell cap and the withdrawal port within the receptor compartment were tightly closed with parafilm.

Receptor phase was stirred constantly throughout the experiment and the temperature kept at 32°C. At set intervals of 0.5, 1, 2, 4, 6 and 8 h 5 ml of the receptor phase was removed and immediately replaced by the same volume of 0.9% w/v sodium chloride solution. The amount of piroxicam released into the receptor phase from gel formulations containing various amounts of penetration enhancers was then calculated by determining the UV absorbance of samples removed at set intervals at 353 nm, using a Shimadzu 120-02 UV-Visible spectrophotometer. For this purpose a standard calibration curve of piroxicam powder (reference standard) in 0.9% w/v sodium chloride solution was constructed, which was found to be linear. The equation of the resulting line was "absorbance=-0.002+0.0455 x concentration" (r = 0.99997). By determining the amount of piroxicam released at various time intervals, the cumulative amount of drug released (µg/cm^2) versus time (h) graphs were plotted. Having plotted the graphs, the linear region of each graph, confirming that a steady state diffusion (permeation) of drug molecules through the skin has been reached, was individually determined. The linearity of this region was carefully tested and confirmed in each case, using statistical testing of linear regression. Having confirmed the linearity of this region, the slope of the line (flux) was calculated. The numerical values of slopes were used for comparison and further calculation of other parameters.

Results and Discussion

Passage of drug molecules through the skin could be an important and rather troublesome stage in percutaneous drug delivery. Among the various methods for improving the passage of drug molecules through the skin is the use of penetration enhancers. In this study the effect of various penetration enhancers on the absorption of piroxicam through rat abdominal skin was evaluated. Piroxicam was chosen as a lipophilic model of a drug molecule. It has a molecular weight of 331.35 and an octanol in water partition coefficient of 1.8 (18). It is stated in the literature that highly lipophilic drugs with partition coefficients greater than 2 or 3 tend to remain in the stratum corneum for an extended period of time and hence will not penetrate well into the lower skin layers, and hence their therapeutic effects will be under question (19).

The effect of OA in amounts of 0.3, 1 and 5% w/w within the piroxicam gel on the permeability rate of drug through rat skin is shown in Figure 1. Slopes of the linear regions of all four lines obtained were then calculated as mentioned before. OA is a popular penetration enhancer and penetrates into the stratum corneum and decompresses this layer and hence reduces its' resistance to drug penetration (20). OA can also accumulate within the lipid bilayers of stratum corneum cells and hence increase their flow ability and penetration ability (21). From the results obtained, the presence of 1% OA seems to have the greatest effect on the permeability rate (slope of the lines obtained) of piroxicam. In fact the permeability rate of piroxicam gel containing 1% OA is significantly (one-way analysis of variance, $P<0.05$) greater than the other two concentrations studied. It is speculated that at a 5% OA concentration, presence of a large amount of this fatty acid could slow down the partitioning of piroxicam out of the gel base and stratum corneum, and hence result in the decrease in permeability rate of piroxicam observed.

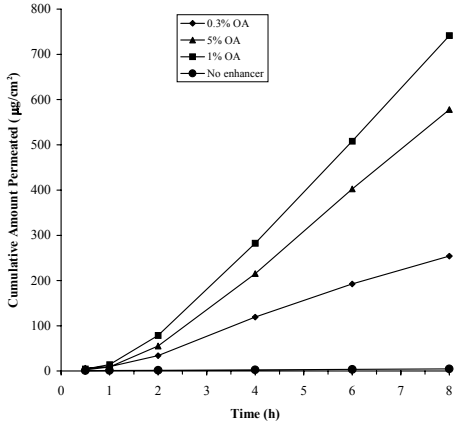

Figure 1. Cumulative amount of piroxicam permeated through sections of excised rat skin at 32°C, from a 0.5%w/w piroxicam gel containing 0.3, 1.0 and 5.0%w/w OA (n=3).

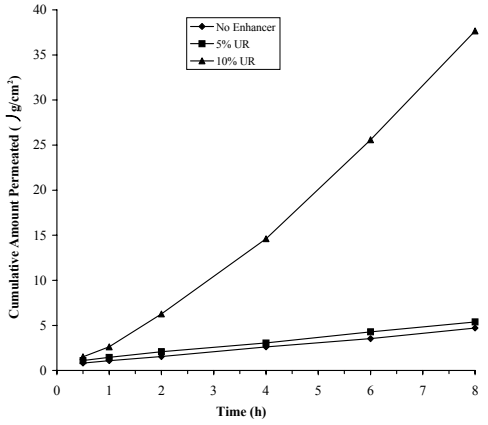

Figure 2. Cumulative amount of piroxicam permeated through sections of excised rat skin at 32°C, from a 0.5%w/w piroxicam gel containing 5 and 10%w/w UR (n=3).

Next, the effect of UR in concentrations of 5 and 10% w/w within the gel, on the permeability rate of piroxicam through rat skin, was investigated. Results obtained are shown in Figure 2. The slopes of the linear regions of all three lines were calculated as mentioned earlier. UR is among the amide compounds and is widely used in amounts of around 5-10% in moisturizing creams and topical steroids (20). UR is a hygroscopic compound, which hydrates the stratum corneum and hence improves permeation of drug molecules. By increasing the amount of water present within stratum corneum, this layer swells and even though the passageway of drug molecules gets longer but nevertheless because of the decompression of cells and an increase in the rate of passage of drug molecules, the extent of drug permeation increases (12, 22). When using a concentration of 5% w/w UR in the piroxicam gel, the permeability rate of drug was only found to be slightly more than the gel containing no enhancer. This finding means that at a concentration of 5%w/w, UR only slightly swells and decompresses stratum corneum, but not to a desirable extent in order to allow quick movement of piroxicam molecules across the skin. In contrast, when increasing the amount of UR present within the gel formulation from 5 to 10% w/w, the permeability rate of piroxicam was found to be significantly ($P<0.05$, student's t-test) greater than the gel containing only 5% w/w UR. This would mean that a UR concentration of 10% w/w can be effective as a moderate penetration enhancer for increasing the permeability rate of piroxicam. However, nevertheless it should be noted that this study suggests that the permeation enhancement ability of UR on piroxicam is greatly less than all OA concentrations used in this study. This is probably due to the fact that UR mainly increases the extent of swelling of stratum corneum and this will be more beneficial for the hydrophilic drugs (8), and to a lesser extent for the lipophilic molecule of piroxicam.

The third penetration enhancer used in this study was LEC. This agent was incorporated within the piroxicam gel at concentrations of 1, 5 and 10% w/w. The results obtained from assessing the effect of this agent on the permeability rate of piroxicam through excised rat skin is shown in Figure 3. Again the linear regions of the curves obtained in Figure 3 were determined as before. LEC or phosphatidyl choline contains fatty acids and has surfactant properties and could affect the lipids of stratum corneum, altering their arrangement and disordering them, hence increasing drug permeation and in particular lipophilic molecules such as piroxicam (23, 24). Results obtained from this study show that at a 5% w/w LEC concentration, the greatest permeability rate of piroxicam is achieved. However, at a LEC concentration of 10% w/w a significant reduction ($P<0.05$, student's t-test) in permeability rate of piroxicam through excised rat skin, in comparison with the 5%w/w LEC concentration, was noted. This finding could possibly be due to extensive complex formation between piroxicam and LEC molecules at high LEC concentrations, making the partitioning of piroxicam molecule out of gel base rather difficult, hence reducing the amount of drug molecules permeated through the skin. It should also be noted that the permeation enhancement ability

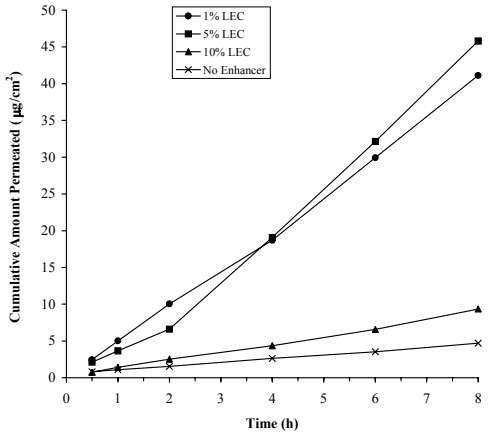

Figure 3. Cumulative amount of piroxicam permeated through sections of excised rat skin at 32ºC, from a 0.5%w/w piroxicam gel containing 1, 5 and 10%w/w LEC (n=3).

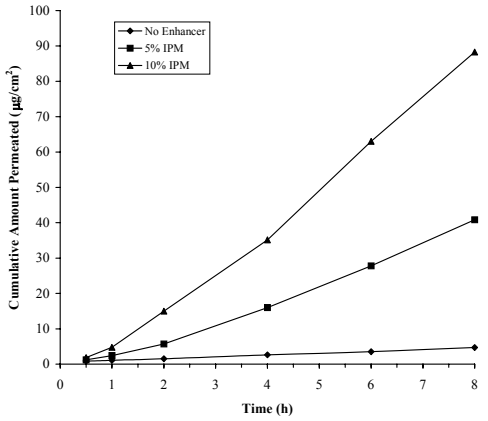

Figure 4. Cumulative amount of piroxicam permeated through sections of excised rat skin at 32ºC, from a 0.5%w/w piroxicam gel containing 5 and 10%w/w IPM (n=3).

of LEC at concentrations of 1 and 5% w/w on piroxicam gel is even though slightly greater than UR (when used at a concentration of 10%w/w), but still greatly less than all OA concentrations used in this study.

The final penetration enhancer evaluated was IPM. This agent was added in concentrations of 5 and 10% w/w to piroxicam gel. Concentrations greater than 10%w/w would not mix well with the gel base and hence the use of IPM was limited up to an amount of 10% w/w in formulation. The results obtained are shown in Figure 4. IPM is an aliphatic ester, which is widely used as a safe penetration enhancer in dermatological formulations. It's mechanism of action is not precisely understood, but it seems that IPM penetrates between the lipid bilayers of stratum corneum and due to its' chain structure, disrupts the order and arrangement of lipid bilayers of stratum corneum and hence improves drug permeation into this layer (25). It should also be noted that IPM has a synergistic effect in the presence of propylene glycol (26), and since propylene glycol is present in this gel formulation, it is expected that its' permeation enhancement effect gets magnified. Results show that the use of IPM at a concentration of 10% w/w has a significantly ($P<0.05$, student's t-test) greater effect on the permeability rate of piroxicam through excised rat skin than the 5% w/w concentration. In fact it seems that the permeability rate of piroxicam doubles as a result of increasing IPM concentration from 5 to 10% w/w in the formulation. Finally, based on this study the permeation enhancement ability of IPM on piroxicam appears to be greater than UR and LEC, but significantly ($P<0.05$, one-way analysis of variance) less than all the various concentrations of OA used in this study.

In the final part of this study attempts were made to compare the "enhancement ratio (ER)" of various penetration enhancers used. This is a ratio of permeability coefficient constant (k_p) following the use of penetration enhancer, divided by the permeability coefficient constant before the use of penetration enhancer (k_p after / k_p before). The greater the ER, the greater the permeation enhancement ability of penetration enhancer used.

In order to calculate ER of various concentrations of penetration enhancers used, the slope of the accumulative amounts of piroxicam permeated at different time intervals through excised sections of rat skin were calculated from Figures 1-4. This parameter is the permeability rate or flux. Using this parameter k_p (cm/h) was calculated, based on Fick's first law of diffusion. In here the total amount of drug (piroxicam) present was 9000 µg, since as mentioned earlier 300 mg of a 0.5% w/w piroxicam gel was spread over each cm^2 of skin, and the overall area of skin used for spreading the gel was 6 cm^2.

The ER of various concentrations of penetration enhancers used in this study is summarized in Table 1. As can be seen, OA in all concentrations used has the greatest ER among the penetration enhancers examined. In fact, based on this study OA appears to be the best penetration enhancer for increasing the permeability rate of piroxicam. Overall, the addition of OA at a concentration of 1% w/w to the piroxicam gel appears to provide the greatest drug permeability rate and ER. This finding suggests that the type and

Table 1. Enhancement ratio of various amounts of penetration enhancers incorporated within a 0.5%w/w piroxicam gel, when placed in contact with excised sections of rat skin at 32°C

Amount of penetration enhacer used (%w/w)	Enhancement ratio
0.3 % OA	68.5 ± 8.1
1.0 % OA	193.1 ± 42.3
5.0 % OA	74.2 ± 10.5
5.0 % IPM	10.4 ± 4.1
10.0 % IPM	22.6 ± 9.3
1.0 % LEC	9.4 ± 0.7
5.0 % LEC	11.5 ± 2.0
10.0 % LEC	2.2 ± 0.8
5.0 % UR	1.1 ± 0.1
10.0 % UR	9.4 ± 1.6

concentration of a penetration enhancer for incorporation into a specific formulation containing a particular drug should be selected carefully and following extensive initial studies, in order to achieve a formulation with desirable drug permeability rate and efficacy.

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