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Mild Local Hyperthermia Enhances the Transdermal Permeation of Ketotifen

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The permeation of ketotifen fumarate across rat skin was studied at 37 and 40 °C. The flux was enhanced at 40 °C by 18 fold over 37 °C. Diffusion studies across dialysis membrane and stripped skin at two temperatures revealed that the primary mechanism of enhancement at mild hyperthermia conditions is most likely due to the alteration of physicochemical nature of stratum corneum lipids and additionally due to the increased kinesis of molecules. The results show how an increase in temperature by a few degrees above physiological temperature increases the permeation of hydrophilic drugs.

Key Words: Hyperthermia, Transdermal permeation, Ketotifen.

INTRODUCTION

Ketotifen is one of the potent drugs used in the treatment of asthma. It is preferably used to help prevent asthma attacks than to relieve an asthma attack. It must be taken continuously in order to be effective. The dose in adults and children is 2 mg/day in divided dose. It is substantially metabolized in the liver when administered orally[1]. From the pharmacokinetic perspective, it is an ideal candidate for formulation of transdermal drug delivery systems for prolonged therapeutic activity in chronic asthmatic patients. The transdermal drug delivery of ketotifen is also believed to help in overcoming the nocturnal attacks in the patients by maintaining constant therapeutic levels[2]. Ketotifen is hydrophilic and the skin penetrability for ketotifen is poor. Hence it is a drug of interest to study the effect of transdermal penetration enhancers.

Stratum corneum is the actual barrier for the transdermal drug absorption consists of several layers of dead keratinized cells. The intercellular space is occupied by the saturated lipids consisting of ceramides, cholesterol and

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fatty acids. The lipid mixture is reported to possess four transition temperatures, of which the first transition occurs at mild hyperthermia conditions (*ca.* 40 °C). At this temperature, the permeability of the skin is known to increase for the diffusion of hydrophilic drugs[3]. In this study we assessed the influence of slight increase in the skin surface temperature by a few degrees above the normal physiological temperature on the permeation of ketotifen fumarate (procured from Sigma Aldrich, USA).

EXPERIMENTAL

The *in vitro* diffusion studies were carried out using a Franz diffusion cell with an active diffusion area of 0.64 cm^2. Freshly excised rat skin was used as the diffusion barrier for *in vitro* permeation studies. Sprague dawley rats were sacrificed by carbon dioxide asphyxiation and the dorsal and flank area was shaved using fine clippers, taking care to avoid damaging the skin. The area was carefully excised and any underlying fat or muscle tissue was removed. The skin was cut into required size and used immediately. Rat skin was sandwiched between donor and receiver compartments with the stratum corneum part facing the donor compartment. The barrier integrity of rat skin was confirmed prior to transport studies, by measuring the electrical conductance at 37 °C using two 4 mm Ag/AgCl disk electrodes introduced into the diffusion cell, one in the receiver compartment and the other in the donor compartment of the diffusion apparatus[4]. The skin samples having a resistivity > 30 kΩ cm^2 were only used for the transport studies. Both the compartments were filled with phosphate buffer saline (PBS) for resistance measurements. The temperature was maintained by water circulation through the diffusion cell. During the transport studies, the receiver compartment was filled with PBS, whereas the donor compartment was filled with drug solution (25 mM) prepared in PBS (control). The receiver compartment buffer was withdrawn at different time intervals to measure Ketotifen. Ketotifen was measured by HPLC with a UV detector at 301 nm[5]. The column used was C18 reverse phase and the mobile phase was methanol, water and phosphoric acid (750:1250:1 v/v) at a flow rate of 0.5 mL/min. The detection limit of ketotifen was 0.1 μg/mL and the coefficient of variation of the method was 4.1 %.

For some specific experiments involving the mechanistic study of temperature influence, cellophane membrane or stripped skin (stratum corneum was removed by 30 times tape stripping of skin) was used as the barrier in the diffusion studies instead of the full thickness rat skin.

The data in the graphs represents the mean readings of 6 trials with the error bars representing the standard deviation. The t-test was selected as the test for significance and P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The transdermal flux of ketotifen across rat skin was calculated from the linear portion of the diffusion profile (Fig. 1). At 37 °C, the diffusion flux was 1.57 ± 0.12 μmol/cm^2 which was enhanced to 28.97 ± 3.36 μmol/cm^2 at 40 °C. The lag time was however not significantly different. At this stage it was not certain whether the enhanced permeation of ketotifen was due to the influence of temperature on the barrier properties of the skin or on the kinesis of molecule. Diffusion studies across the dialysis membrane (Fig. 2) were carried out at two temperatures 37 and 40 °C. As there could be no alteration of barrier properties of the membrane, the temperature effect on mere diffusion kinetics could be assessed. The enhancement factor across the dialysis membrane at 40 °C was *ca.* 4 fold. However across the rat skin, the enhancement factor at 40 °C was *ca.*18 fold. Therefore it is evident that increase in temperature from 37 to 40 °C has increased the permeation of drug predominantly by altering the barrier properties of the skin.

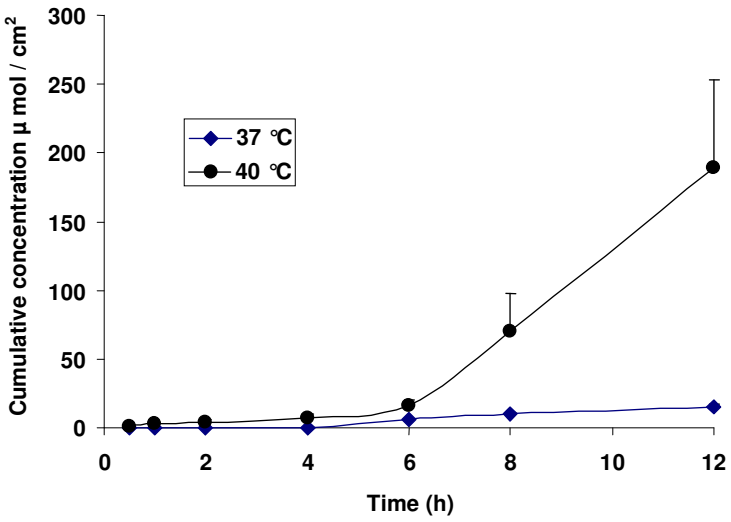

Fig. 1. Transdermal permeation of ketotifen across rat skin from 25 mM ketotifen solution prepared in PBS (♦) 37 °C (•) 40 °C

Transdermal diffusion studies of ketotifen was also carried out across tape stripped (no stratum corneum) skin. The flux at 37 °C was 81 ± 14.35 μmol/cm^2 and at 40 °C 288.37 ± 46.90 μmol/cm^2. The enhancement factor at 40 °C across the stripped skin was *ca.* 3.7. This is comparable with that found across the dialysis membrane. From these results, it is evident that

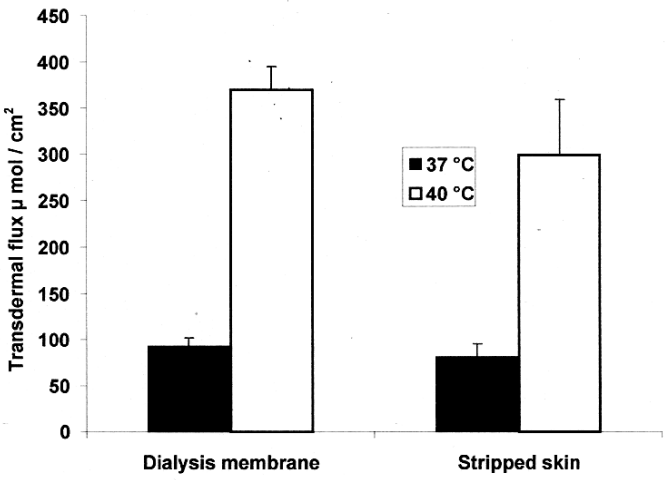

Fig. 2. Transdermal diffusion flux of ketotifen fumarate across dialysis membrane (3500 kDa cut off mol. wt) and stripped skin at (■) 37 °C (□) 40 °C

the stratum corneum is the predominant barrier for the diffusion of ketotifen fumarate. It is also likely that the viable tissue layers and dermal tissue cells offer the same resistance as that of water for diffusion of the drug. Therefore similar transdermal flux, as well as enhancement ratios were observed in case of dialysis membrane and stripped skin at the two temperature conditions. It is most likely that the primary influence of increased temperature on the skin is on the stratum corneum layer. Stratum corneum consists of closely packed keratinized cells, through which the absorption of drug is relatively negligible. The lipids present in the intercellular space are impermeant as well for the hydrophilic molecules. However, altering the physicochemical nature of these lipids renders these domains relatively permeable to hydrophilic drugs. This could be the reason for synergistic enhancement in penetration of ketotifen at 40 °C over 37 °C. It is presumed that the lipids attain a more dynamic state at 40 °C thus favouring hydration of lipid bilayers. This facilitates the absorption of ketotifen fumarate.

This study demonstrates that temperature not only influences the diffusant's kinetic properties but also leads to alteration of physico-chemical nature of the lipid bilayer domains in the intercellular space in stratum corneum. The increase in temperature by 3 °C above the physiological temperature can help is increasing the permeation of Ketotifen. It may be speculated that such similar effect may be seen with other hydrophilic drugs

also. Increasing the local temperature of skin at the site of application of a transdermal patch may not be a difficult task. Heat patches, thermal pads *etc.*, may be used to permeabilize the stratum corneum for permeation of relatively greater amounts of hydrophilic drugs.

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