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STUDY OF PERCUTANEOUS ABSORPTION OF DICLOFENAC DIETHYLAMINE IN THE PRESENCE OF CETRIMIDE THROUGH HAIRLESS RABBIT SKIN

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Abstract: In the present study, the effect of Cetrimide as an enhancer on transdermal absorption of 1% diclofenac diethylamine (Non-steroidal Anti-inflammatory Drug) through hairless rabbit skin was evaluated in-vitro study at various concentrations to improve the skin permeability. From the data, Cetrimide shows the small lag time which gives a picture about its enhancing effect. The permeability co-efficient and flux rate calculated for diclofenac diethylamine in the presence of Cetrimide shows that the penetration of drug through hairless rabbit skin has been significantly increased.

Keywords: Diclofenac, enhancer, skin permeability, topically, transdermal.

INTRODUCTION

Transdermal drug delivery could provide constant drug release for days, avoids first-pass metabolism, and could allow drug effects to be rapidly terminated by simply removing the transdermal patch. However, absorption across the skin for molecules larger than 1000 Daltons has proved to be difficult, even with the addition of permeation enhancers [Rodney *et al*. 2003]. The amount of drug bioavailable for targeting the sites of action is lower than via oral route, but the absorbed dose appears to be adequate for therapeutic use [Katz and Poulsen 1971].

Poor absorption of small-molecule drugs across the stratum corneum has been presumed to be due to the drug's physio-chemical properties, which can be systematically modified by medicinal chemists, and the nature of the physiological barrier, which can sometimes be transiently altered. To overcome physiological barriers, scientists have studied a series of synthetic and natural compounds exhibiting absorption enhancement properties. These molecules, included in the dosage formulation to enhance absorption, are generally known as permeation enhancers, which are categorized into four classes (I, II, III & IV), according to their effectiveness and safety [Muranishi 1990 and Aungst 2000].

Various Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs worldwide and are responsible for approximately quarter of all adverse drug reports. They are widely prescribed for patients with rheumatic disease with a popular increased risk for serious gastrointestinal tract complications. These NSAIDs with proven efficacy by oral route of administration has been investigated for efficacy in topical dosage forms [Roberts II and Morrow 2001].

MATERIALS AND METHODS

CHEMICALS

Diclofenac diehtylamine supplied by Novartis (China origin), the enhancer used was Cetrimide (Merck). Double distilled water from an electrically heated still, having the pH 6.8 determined by the pH meter and stored in a well leached amber glass bottle, was used throughout the experiment. Ethanol (Merck), sodium acetate (Merck) and sodium chloride (Merck) were used. HPLC was used to analyze the sample taken using column C_{18}, 5 µm, 150 mm length, 4.5mm internal diameter (Neocleocil; Alltech) and pump WATER'S 600E, Detector WATER'S 484, with the help of HPLC Software Millennium Version-2.15 (courtesy Novartis Pakistan).

ANIMAL SKIN

In-vitro technique applied to study transdermal absorption involves the use of animal excised skin; in many cases full thickness was used [Hadgraft *et al*. 2003].

ASSAY SOLUTION (MOBILE PHASE)

An isocratic mobile phase was used containing 0.1 M sodium acetate.

CONTROL SOLUTION

1 gram of diclofenac diethylamine was dissolved in 5 ml methanol in 100ml volumetric flask and the volume was made up to the mark with normal saline. This was used as reference control solution without any enhancers.

TEST SOLUTION

Test solution was prepared by dissolving 1 gram of diclofenac diethylamine in 5 ml methanol in 100 ml volumetric flask and the solution was made up to the mark with previously constituted solutions of 0.1, 0.2, 0.3, 0.4, 0.5 and 1% enhancer (Cetrimide) in normal saline.

DIFFUSION CELL

Diffusion cell was fabricated after Franz [1975], Keshary [1984], Tojo [1985], Cordero *et al*. [1996], Takahashi [1996] and Valenta [2000] with some modifications. The cell was in the form of two cylindrical glass half cells. The inside diameter was 2 cm. The diffusion cell halves were termed as upper (donor compartment) and lower (receptor compartment) half cells. The volume capacity of the donor and receptor compartments was 40ml and 35ml, respectively. The membrane was mounted in between the two half cells and the exposed penetration area was approximately 3.14cm^2. From the lower half of the receptor compartment at a distance of about 3.8 cm a side arm 4 cm in length was used for taking the sample and correcting the volume of receptor compartment with the help of saline solution by exposing the epidermal side towards

the donor half cell. The two half cells after clamping were mounted on a magnetic stirrer and small magnetic flea was placed in the receptor compartment and the receptor solution was stirred at ~60 rpm.

MEMBRANE PREPARATION

The membrane, full thickness skin, was taken from the abdominal surface of the hairless rabbit. The skin at the lower abdomen was marked, shaved and sacrificed. The whole skin was then removed and a rectangular section marked was excised from the animal with surgical scissors. As the skin was not firmly attached to the viscera it was lifted easily from the animal after the incision was made. Prior to the skin removal, a uniform circle was made on the abdomen, marking the precise skin section to be positioned between the two half cells after the excised skin was trimmed into an oversized rough circle it was mounted between the half cells with the marked section centered. The skin was placed in a normal saline solution before mounting onto the diffusion cell [Durrheim *et al*. 1980, Cordero *et al*. 1996].

Skin was cut according to the diameter of the diffusion cell. The half cell was held fast by a clamp stretching the skin off as evidenced by distortion or expansion of the circular outline, which was corrected.

CHARGING THE CELL

The receptor cell filled with normal saline was stirred by magnetic stirrer at 60 rpm for 30 minutes, at which time the compartments were evacuated with a syringe and refilled with fresh normal saline. Then the compartments were evacuated, a second time refilled, evacuated a third time and finally refilled with normal saline. The donor compartment of the cell was charged with a test solution.

PERMEATION

The donor compartment of the cell was charged with a test solution containing 1% of diclofenac diehtylamine plus different concentrations of enhancer dissolved in 100ml of normal saline. The receptor cell contents were stirred and at predetermined times samples were taken and transferred to the small bottles having stoppers using 10 ml syringe. The time of charging the donor compartment was noted at the beginning of the diffusion runs and the receptor samples were referenced to this time.

SAMPLING

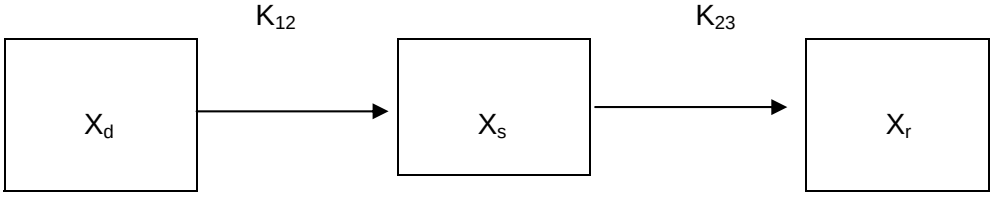
From the side arm of the receptor compartment, 5 ml of the sample was drawn each time at an interval with the help of 5 ml syringe and correcting the receptor half cell volume with normal saline. The 5 ml sample was drawn at an interval of 5 minutes for 30 minutes.

A portion of 10 microlitres of the sample taken from the receptor cell was collected and run on the HPLC having a column C_{18} (Neocleocil, Alltech),

pump (WATER'S 600 E), detector (WATER'S 484) using the HPLC Software Millennium Version-2.15 at a flow rate of 0.8ml per minute, by using auto sampler at the wavelength of 254nm.

PHARMACOKINETIC ANALYSIS OF IN-VITRO TRANSDERMAL PENETRATION

Data of the in-vitro transdermal absorption study using stripped skin were analyzed on the basis of Pharmacokinetic model as shown below:



Donor Cell **Skin Cell** **Receptor Cell**

RESULTS AND DISCUSSION

The permeation profile of the receptor phase concentration in microgram per 100ml is summarized in Table 1. The lag time of the plots was calculated graphically by extrapolating from pseudo-steady-state region of the plot between total amount penetrated and time to the x-axis. The diffusion coefficients and permeability coefficients of different concentrations of enhancer were calculated as follows:

The diffusion coefficient (D) was calculated by dividing square of the thickness of rabbit incised skin 'h' by 6 x lag time 'L' [Badar 1992]:

$$D = \frac{h^2}{6L} \quad (cm^2\ h^{-1} \times 10^{-n}) \qquad (1)$$

Permeability coefficient (P) was calculated by taking the ratio of diffusion coefficient and square of the effective absorption area 'A' of the skin in contact [Tsai *et al*. 2001]:

$$P = \frac{D}{A^2} \quad (cm\ h^{-1} \times 10^{-n}) \qquad (2)$$

Table 1: Effect of different concentrations of Cetrimide on the permeability parameters of l% diclofenac diethylamine using rabbit skin.

Concentration. of Enhancer (%)	Permeation Co-efficient (P)	Flux (J)	Diffusion Co-efficient (D)	Rate Constant	ER
0.1	0.000514	0.0608	0.003030	0.81901	2.971098
0.2	0.000614	0.0212	0.003617	0.92613	3.549132
0.3	0.000529	0.0187	0.003114	0.68036	3.057803
0.4	0.001119	0.0792	0.006596	0.81901	6.878612
0.5	0.000544	0.0769	0.003203	1.07891	3.144508
1.0	0.001844	0.6909	0.001844	0.90012	10.65895

When value of permeation coefficient (P) for sample without enhancer was 0.000173

Enhancement ratio (ER) which measures the penetration enhancing activity of the enhancers was calculated as follows:

$$ER = \frac{P \text{ after application of penetration enhancer}}{P \text{ before application of penetration enhancer}} \quad (3)$$

K_P is the mean permeability coefficient of drugs [Abdullah *et al*. 1996]. Typical results have been shown in Table 1. The data was subjected to proper relevant statistical analysis.

The Flux (J) of a drug measures the mass of material transported through the skin so is more relevant parameter, therapeutically, than permeability coefficient [Rautio 1999]. It is directly proportional to the thermodynamic activity of a drug, i.e.:

$$J = - D \times \frac{dc}{dx} \qquad (\mu g\ cm^{-2}\ h^{-1}) \quad (4)$$

These values of flux indicate that penetration may be dependent on the lipoidal solubility of drug moiety. However, permeation may be complicated by charge effect and it may also depend on the skin partition coefficient of the drug between aqueous and lipid phases of the barrier [Shah *et al*. 2005).

$$\text{Partition Coefficient (Pc)} = \frac{P}{-D} \qquad (x10^{-n}) \quad (5)$$

The diffusion coefficient presented in Table 1 reflect their effects on permeability coefficient of diclofenac diethylamine. The charge in lag time changes the diffusion coefficients of diclofenac diethylamine which increases with decrease in lag time [Aguiar 1969, Durrheim *et al*. 1980].

Finally, the permeability rate constants were calculated at various concentrations of enhancers and were summarized in Table 1. As the whole penetration process was assumed to be first order rate constant, the rate constant could thus be calculated as under [Badar 1992]:

$$\text{Rate Constant} = \frac{\text{Log}\ (y_2 - y_1)}{t_2 - t_1} \times 2.303 \quad (6)$$

CONCLUSIONS

Cetrimide shows different behaviour as permeability rate constant of 1% diclofenac diethylamine remains almost same at the concentrations of 0.1 and 0.2% of Cetrimide and then increases gradually up to 0.5% and then again remains the same.

In the present study, the effect of Cetrimide on transdermal absorption of diclofenac diethylamine through hairless rabbit skin was evaluated at various concentrations. From the data, following points are concluded:

1. In the interpretation of results the lag time plays an important role. Cetrimide shows that the smaller lag time gives a picture about its rapid enhancing effect as compared to samples without Cetrimide.
2. The permeability coefficient calculated for diclofenac diethylamine under the influence of Cetrimide shows better enhancing characteristics.

3. The rate constant shows fluctuations at various time intervals.
4. The flux rate of diclofenac diethylamine in the presence of Cetrimide shows that the penetration of drug through hairless rabbit skin almost increased.

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