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IN-VITRO STUDY OF PERCUTANEOUS ABSORPTION OF DICLOFENAC IN THE PRESENCE OF SODIUM LAURYL SULPHATE THROUGH RABBIT SKIN

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Abstract: In the present study, effect of various concentrations of Sodium Lauryl Sulphate (SLS) as an enhancer on transdermal absorption of Diclofenac diethylamine through hairless rabbit skin was evaluated in-vitro.
From the data, SLS shows that the smallest lag time gives a picture about its rapid enhancing effect. The permeability co-efficient and flux rate calculated for Diclofenac in the presence of SLS as an enhancer shows that the penetration of drug through hairless rabbit skin has been significantly increased.

Keywords: Diclofenac, enhancer, topically, transdermal.

INTRODUCTION

In theory, transdermal 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 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 and Milo 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 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 and 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

MATERIALS AND CHEMICALS

Diclofenac Diehtylamine was supplied by Novartis (China origin), the enhancer used was sodium lauryl sulphate (E. 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 (E. Merck) and sodium acetate (E. Merck) were also used.

High performance liquid chromatography (HPLC) was performed for the analysis of the sample taken using column C18, 5 micrometer, 150 mm length, 4.5mm internal diameter (Neocleocil; Alltech) and pump WATER'S 600E, Detector WATER'S 484 using the HPLC software Millennium Version-2.15 (courtesy Novartis Pakistan).

ANIMAL SKIN

An in-vitro technique used to study transdermal absorption involves the use of animal excised skin; in many cases full thickness is used but in this study the full skin of the rabbit was used.

ASSAY SOLUTIONS (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 solutions were prepared by dissolving 1 gram of diclofenac diethylamine in 5 ml methanol in 100 ml volumetric flasks and the solutions were made up to the mark with previously constituted solutions of 0.5 and 1% enhancer (Sodium Lauryl Sulphate) in normal saline.

DIFFUSION CELL

Diffusion cell was fabricated after Franz [1975], Keshary [1984], Tojo [1985], Cordero [1996], Takahahsi [1996], 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 half cell (donor compartment) and the lower half cell (receptor compartment). The volume capacities of the donor and receptor compartments were 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 is

used for taking the sample and correcting the volume of receptor compartment with the help of saline solution by exposing the epidermal side toward the donor half cell. The two half cells after clamping were mounted on a magnetic stirrer and small magnetic fleas were placed in the receptor compartment, and the receptor solution is stirred at 60 rpm.

MEMBRANE PREPARATION

The membrane, full thickness skin taken from the abdominal surface of the hairless rabbit. The skin at the lower abdomen was marked and was shaved and then rabbit skin was sacrificed and whole skin was removed and a rectangular section marked was excised from the animal with surgical scissors. Since 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 on to the diffusion cell [Durrheim *et al*. 1980, Cordero 1996].

Skin was cut according to the diameter of the diffusion cell and the half cells was held fast by a clamp stretching of the skin as evidenced by distortion or expansion of the circular outline was corrected and the half cells were held fast by a clamp.

Charging the Cell

The receptor cell contents were stirred by magnetic stirrer at 60 rpm the receptor compartment was filled with normal saline and stirred 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 compartments of the cell were 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, sample 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 reference to this time.

Sampling

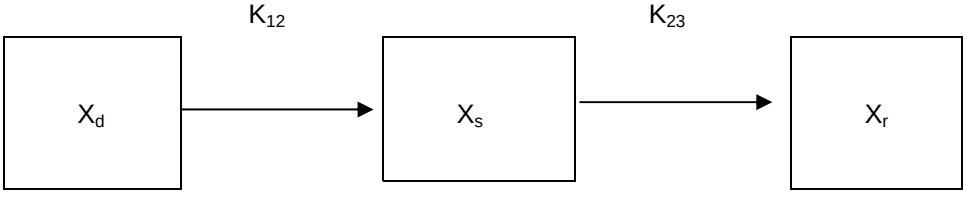
From the side arm of the receptor compartment 5 ml of the samples were drawn with the help of 10 ml syringe and correcting the receptor half cell

volume with normal saline. 5 ml sample is drawn at an interval of 5 minutes for 30 minutes.

A portion of 10 microlitres of the sample, taken from the receptor cell, was run on the HPLC having a column C18 (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 shown in following Fig.



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 extrapolation from the pseudo steady state region of the graph of total amount penetrated versus time to x-axis.

Table 1: Effect of different concentrations of SLS on the permeability parameters of l% Diclofenac diethyl amine using rabbit skin.

Concentration of Enhancer (SLS) (%)	Permeation Co-efficient (P) (cm^2. h^{-1} x 10^{-n})	Flux (J)	Diffusion Co-efficient (D) (cm^2. h^{-1} x 10^{-n})	Rate Constant	Enhancement Ratio
0.1	0.000373	0.1838	0.002198	1.002308	2.156
0.2	0.004758	0.1406	0.028033	0.680362	27.50
0.3	0.000344	0.1427	0.003398	1.069614	1.980
0.4	0.001586	0.0562	0.009344	0.578178	9.167
0.5	0.000317	0.0093	0.001868	0.680362	1.830
1.0	0.001903	0.0187	0.011213	0.321945	11.00

When value of Permeation co-efficient (P) for sample without enhancer was 0.000173

The Diffusion Coefficients of different concentrations of enhancer were calculated by dividing square of the thickness 'h' of the rabbit incised skin by 6 x Lag time 'L' [Badar 1992]:

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

The Permeability Coefficient (P) was calculated by dividing the Diffusion Coefficient D by square of the effective absorption area 'A' of the skin in contact [Tsai 2001]:

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

and K_P is the mean permeability coefficient of drug [Abdullah *et al*. 1996].
As a measure of the penetration enhancing activity of the enhancers the enhancement ratio (ER) was calculated as:

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

The Flux of a drug is directly proportional to its thermodynamic activity:

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

Flux measures the mass of material transported through the skin and hence is more relevant parameter, therapeutically, than the Permeability Coefficient [Rautio 1999].
All these values indicate that penetration may be dependent on lipoidal solubility of the drug moiety. However, the permeation may be complicated by charge effect and also may depend on the skin Partition Coefficient of the drug between the aqueous phase and lipid phase of the barrier. The Partition Coefficient was calculated by the relation:

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

The Diffusion Coefficients presented in Table 1 reflects its effects on Permeability Coefficients of Diclofenac. The charge in Lag time changes the Diffusion Coefficients of Diclofenac that increases with decrease in Lag Time [Aguiar and Weiner 1969, Durreheim *et al*. 1980].
Finally the permeability rate constant was calculated at various concentrations of enhancer, which are summarized in Table 1. It was assumed that the whole penetration process is first order rate constant, so the rate constants can be calculated using following expression:

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

SLS shows different behaviour as permeability rate constant of 1% Diclofenac decreases from 0.1% to 0.2% and then after showing a slight increase it again decreases up to 1%.

CONCLUSIONS

In the present study, effect of SLS on transdermal absorption of Diclofenac through hairless rabbit skin was evaluated at various concentrations. From the data, following points were concluded:

1. The Lag time plays an important role in the interpretation of results. SLS indicates that the smallest Lag time gives a picture about its rapid enhancing effect as compared to samples without SLS.

2. The permeability coefficient calculated for Diclofenac under the influence of SLS shows better enhancing characteristics.
3. The flux rate of Diclofenac in the presence of SLS shows that the penetration of drug through hairless rabbit skin has almost been increased.
4. The rate constant shows fluctuations at various time intervals.

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