

EFFECT OF UREA ON TOPICAL ABSORPTION OF DICLOFENAC DIETHYLAMINE THROUGH HAIRLESS RABBIT SKIN

**Syed Nisar Hussain Shah*, Mahboob Rabbani
and Muhammad Fakhruddin Amir**

Faculty of Pharmacy, Bahauddin Zakariya University, Multan-Pakistan.

Abstract

In the present study, the effect of Urea 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, Urea shows slightly large lag time gives a picture about its enhancing effect. The permeability rate constant is almost approaching to 1 in almost all the concentrations of urea. The permeability co-efficient of Diclofenac Diethylamine did not change under the influence of urea at concentration 0.1, 0.2 and 0.3% but it was increased by increasing the concentration of urea.

Keywords: Diclofenac, enhancer, topically, transdermal.

INTRODUCTION

In the severe renal failure Urea can create precipitates on the skin surface. It is also possible to find increased urea concentration in the skin layers. Urea is a physiological component of NMF (natural moisturizing factor) of the skin. Owing to its mild keratolytic effect, urea is used in the topical drug formulations. Urea and its analogues were also studied as possible candidates to accelerate percutaneous absorption of drugs [Wong *et al.* 1988, Williams and Barry 1989].

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 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 Gibaldi 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].

Many 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 [Roberts II and Morrow 2001]. They are used in the treatment of osteo- and rheumatoid arthritis as well as local inflammation. [Roberts 1999, Hadgraft *et al.* 2000]. The well-known side effects observed after oral administration of NSAIDs have accelerated the development of alternative

pharmaceutical forms such as topical cream, gel, lotion, foam and Transdermal-patch formulations that allow local absorption at the inflammation site without adverse systemic reactions [Rhee *et al.* 2001, Djordjevic 2003].

MATERIALS AND METHODS

CHEMICALS

Diclofenac Diethylamine supplied by Novartis (China origin), the enhancer used was Urea (Merck). Double distilled water from an electrically heated still, having the pH $6.8 \pm$ 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 for the analysis of the sample taken using column C₁₈, 5 μ m, 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

In-vitro technique that was used to study transdermal absorption involves the use of animal excised skin; in many cases full thickness is 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 100 ml 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 (Urea) in normal saline.

DIFFUSION CELL

Diffusion cell was fabricated after Franz [1975], Keshary [1984], Tojo [1985], Cordero *et al.* [1996], Takahashi [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 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 3.14cm². 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 was 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 was 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 *et al.* 1996].

Skin was cut according to the diameter of the diffusion cell and the half cell 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 cell were held fast by a clamp.

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 Diethylamine plus different concentrations of enhancer, which was dissolved in 100 ml 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 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 is drawn at an interval of 5 minutes for 30 minutes.

From the sample taken from the receptor cell, a portion of 10 micro-liters was taken and 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 0.8 ml per minute, by using auto sampler at the wavelength of 254 nm.

PHARMACOKINETIC ANALYSIS OF IN-VITRO TRANSDERMAL PENETRATION

Data of the in-vitro transdermal absorption study using stripped skin were analyzed on the basis of the Pharmacokinetic model shown in Fig. 1.

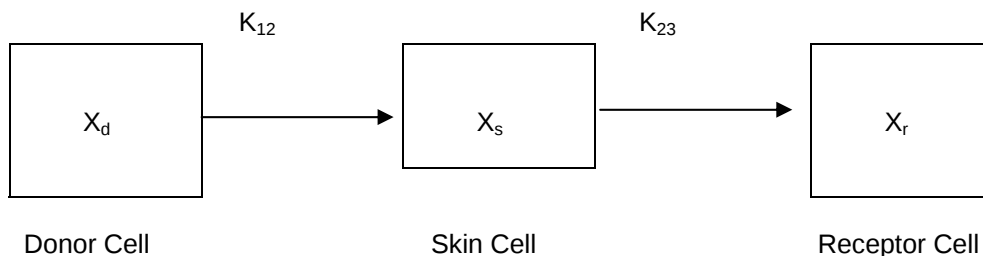


Fig. 1: Pharmacokinetic model for the analysis of in-vitro transdermal absorption study.

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 the total amount penetrated versus time to the X-axis. The Diffusion Coefficients and Permeability Coefficients of different concentrations of enhancer were calculated by dividing the square of the thickness of the rabbit incised skin by 6 x Lag Time [Badar 1992]:

DIFFUSION COEFFICIENT

It was calculated using the following relation:

$$D = \frac{h^2}{6L} \quad (\text{cm}^2.\text{h}^{-1}) \quad (1)$$

where h is the thickness of the rabbit skin and L is the Lag Time.

PERMEABILITY COEFFICIENT (P)

It was calculated by dividing the Diffusion Coefficient by square of the effective absorption area of the skin in contact [Tsai *et al.* 2001]:

$$P = \frac{D}{A^2} \quad (\text{cm}.\text{h}^{-1}) \quad (2)$$

where A is the effective absorption area of the skin in contact.

THE ENHANCEMENT RATIO (ER)

As a measure of the penetration enhancing activity of Urea, the enhancement ratio (ER) was calculated as under [Abdullah *et al.* 1996]:

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

Typical results have been shown in Table 1. The data will be subjected to proper relevant statistical analysis.

Table 1: Effect of different concentrations of Urea on the permeability parameters of 1% Diclofenac Diethylamine using hairless rabbit skin.

Enhancer Concentration	Permeation Co-efficient (P)	Flux (J)	Partition Co-efficient (Pc)	Diffusion Co-efficient (D)	Rate Constant	ER
0.1%	0.000346	0.011	0.169691	0.002039	0.65929	2.000008
0.2%	0.000277	1.226	0.101280	0.002735	1.54330	1.601156
0.3%	0.000247	0.098	0.101313	0.002438	1.07892	1.427746
0.4%	0.000560	0.026	0.169800	0.003298	0.73791	3.236994
0.5%	0.000595	0.077	0.169806	0.003504	0.94027	3.439306
1.0%	0.000669	0.237	0.100450	0.006660	1.03878	3.867052

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

FLUX (J)

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

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

Flux, which measures the mass of material transported through the skin, is more relevant parameter, therapeutically, than the Permeability Coefficient [Rautio 1999].

These values indicate that the penetration may be dependent on the 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 [Shah *et al.* 2005].

PARTITION COEFFICIENT (Pc)

The Partition Coefficient (Pc) can be calculated using the expression:

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

The Diffusion Coefficients presented in Table 1 reflects its effects on Permeability Coefficients of Diclofenac Diethylamine. The change in lag Time, changes the Diffusion Coefficients of Diclofenac Diethylamine that increases with decrease in Lag Time [Aguar and Weiner 1969 and Durrheim *et al.* 1980].

THE PERMEABILITY RATE CONSTANT

Finally, the permeability rate constant of various concentrations of urea was calculated which are also summarized in the same Table 1. As was assumed that the whole penetration process is first order rate constant, the rate constant then can 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

Urea shows fluctuating behaviour as the permeability rate constant is almost approaching to 1 in almost all the concentrations of urea. The permeability co-efficient of Diclofenac Diethylamine did not change under the influence of urea at concentration 0.1, 0.2 and 0.3% but it was increased by increasing the concentration of urea.

From the data, the following points can be concluded:

1. In the interpretation of results the lag time plays an important role. Urea shows the larger lag time gives a picture about its slow enhancing effect as compare to samples without Urea.
2. The permeability coefficient calculated for Diclofenac Diethylamine under the influence of Urea shows enhancing characteristics.
3. The flux rate of Diclofenac Diethylamine in the presence of Urea shows that the penetration of drug through hairless rabbit skin almost increased.
4. The Diffusion Co-efficient of the drug increases all the way up to 1.0% of urea showing the steady increase initially and a sharp one in the end.

Acknowledgement

The researchers greatly acknowledge the co-operation extended by Novartis Pakistan Ltd., Jamshoro, Hyderabad and also Bahauddin Zakariya University for providing financial support for this research project.

References

- Abdullah, D., Ping, Q.N. and Liu, G.J. (1996) "Enhancing effect of essential oils on the penetration of 5-Fluorouracil through rat skin", *Acta. Pharmaceutica Sinica*, **31**(3), 214-221.
- Aguilar, A.J. and Weiner, M.A. (1969) *J. Pharm. Sci.*, **58**, 210.
- Badar, R. (1992) "Effect of various enhancers on transdermal absorption of Triamcinolone Acetonide through hairless mouse skin", M.Phil Thesis, Gomal University, Dera Ismail Khan, Pakistan, Ch. 6, pp. 62-74.
- Cordero, J.A. *et al.* (1996) *J. Pharm.* **86**, 503 .
- Djordjevic, J., Michniak, B., Uhrich, K.E. (2003) "Amphiphilic star-like macromolecules as novel carriers for topical delivery of non-steroidal anti-Inflammatory drugs", *AAPS Pharm. Sci.*, **5** (4).
- Durrheim, H., Flynn, G.L., Hihuchi, W.I. and Behl, C.R. (1980) *J. Pharm. Sci.*, **69**, 781.
- Franz, T.J.J. (1975) *Invest. Dermatol.*, **64**, 190.
- Hadgraft, J., Plessis, J., Goosen, C. (2000) "The selection of NSAIDs for dermal delivery" *Internat. J. Pharm.*, **207**, 31-37.
- Hadgraft, J., Whitefield, M., Rosher, P.H. (2003) "Skin penetration of topical formulations of Ibuprofen 5%: an in-vitro comparative study", *Skin Pharmacol. Appl. Skin Physiol.*, **16**, 137-142.

Katz, M. and Poulsen, B.J. (1971) "Absorption of Drugs Through The Skin, A Hand Book of Experimental Pharmacology", Springer-Verlag, Berlin, p.103.

Keshary, P.R. (1984) *Drug Devel Ind. Pharm* , **10**, 883.

Rautio, J. (1999) *J. Med. Chem.*, **43**, 1489.

Rhee, Y., Chor, J., Park, E., Chi, S. (2001) "Transdermal delivery of ketoprofen using micro emulsions", *Internat. J. Pharm.* **228**, 161-170.

Roberts II, L.J. and Morrow, J.D. (2001) In: Goodman and Gillman (Ed.), *The Pharmacological Basics of therapeutics*, 10th ed., McGraw Hill, Medical Publishing Division, Ch. 27, pp. 687-732.

Roberts, M., Cross, S. (1999) "Percutaneous absorption of topically applied NSAIDs and other compounds: role of solute properties, skin physiology and delivery systems", *Inflammoharmacology*, **7**, 339-350.

Rodney, J.Y. Ho and Gibaldi, Milo (2003) "Biotechnology and Biopharmaceuticals, John Wiley and Sons Inc. Publications, New York, Ch.13, pp. 344-345.

Scharffer, H., Zesh, A. and Stuttgen, G. (1982) "Skin Permeability", New York.

Shah, S.N.H. et al. (2005) "In-vitro study of Percutaneous absorption of Diclofenac in the presence of SLS through hairless rabbit skin", *J. Res. Sci. B.Z. Univ.*, **16**(1), 45-50.

Takahashi, K. (1996) *Biol. Pharm. Bull.*, **19**, 893.

Tojo, K. (1985) *Abstract of APHA/APS 37th National Meeting*, Philadelphia, **46**, p.24.

Tsai, J.C., et al. (2001) *J. Pharm. Sci.*, **90**, 1242.

Valenta, C. (2000) *Internat. J. Pharm.*, **197**, 77.

Williams, A.C., Barry, B.W. (1989) "Urea analogues in propylene glycol as penetration enhancers in human skin", *Internat. J. Pharm.*, **36**, 43-50.

Wong, Ooi, Hungtinton, J., Konichi, R., Rytting, J.H., Higuchi, T. (1998) "Unsaturated cyclic urea, new nontoxic biodegradable penetration enhancers, I: Synthesis", *J. Pharm. Sci.*, **77**, 967-991.