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PERCUTANEOUS ABSORPTION OF DICLOFENAC DIETHYLAMINE IN THE PRESENCE OF DIMETHYL SULFOXIDE

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ABSTRACT

The aim of present study was to evaluate the effect of various concentrations of dimethyl sulfoxide (DMSO) on transdermal absorption of 1% diclofenac diethylamine across full thickness, hairless rabbit skin using modified Franz diffusion cell. The receptor compartment was constantly stirring normal saline solution at 37°C. At set intervals up to 24h, 5ml samples were removed from the receptor compartment and the amount of diclofenac diethylamine permeated through the skin was calculated by the UV absorbance at 276 nm. At all concentrations of DMSO appearance of small lag time indicated its transdermal penetration enhancing effect. The permeability co-efficient and flux rate for diclofenac diethylamine in the presence of DMSO also showed increased penetration through the skin. The 'Benchmark', flux rate of diclofenac under the influence of DMSO at all concentrations was observed to increase the penetration of drug through hairless rabbit skin. The rate constant showed continuous increase at various time intervals. The mode of action of this accelerant may be described by combined process of partition and diffusion, the diffusion process being dominant.

Keywords: Dimethyl sulfoxide, Transdermal absorption, Franz diffusion cell, Partition coefficients

INTRODUCTION

In the last decades, transdermal dosage forms have been introduced for providing a controlled delivery via the skin into the circulation system. The 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). Transdermal drug delivery involves a continuous administration of therapeutic molecules through the skin. It has the advantage of maintaining constant drug plasma levels and improving patient compliance (Brown and Langer, 1988). The amount of drug bioavailable for targeting the sites of action is lower than that via the oral route, but the absorbed dose appears to be adequate for therapeutic use, particularly because of the absence of side effects (Devi and Paranjothy, 1999).

The skin surface is consisted of a highly shiny lipid film of various depths, depending on the location on the body. The stratum corneum, however, is the first barrier and much interest has been shown in the percutaneous absorption of chemicals and drugs that elicit therapeutic and toxicological response following skin contact (Riviere *et al.,* 2009).

A wide range of primary pharmacological actions of dimethyl sulfoxide (DMSO) has been documented in laboratory studies: membrane transport, effects on connective tissue, anti-inflammation, nerve blockade (analgesia), bacteriostasis, diuresis, enhancements or reduction of the effectiveness of other drugs, cholinesterase inhibition, nonspecific enhancement of

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resistance to infection, vasodilation, muscle relaxation, antagonism to platelet aggregation, and influence on serum cholesterol in emperimental hypercholesterolemia. This substance induces differentiation and function of leukemic and other malignant cells. DMSO also has prophylactic radioprotective properties and cryoprotective actions. It protects against ischemic injury. DMSO readily crosses most tissue membranes of lower animals and man. Rammler and Zaffaroni (1967) have reviewed the chemical properties of DMSO and suggested that the rapid movement of this molecule through the skin, a protein barrier, depends on a reversible configurational change of the protein occurring when DMSO substitutes for water. Several studies report the increased skin penetration of drugs in animals and humans when dissolved in DMSO (Amstey and Parkman, 1966; Keil, 1967; Djan and Gunber, 1967; Maibach and Feldmann, 1967; Sulzberger *et al.,* 1967). This statement has been supporting the use of DMSO in this study and considered its worth to use to enhance the skin penetration of the model drug, diclofenac diethylamine.

Diclofenac, {(2-[2,6-dichlorophenyl) armino] phenyl-acetate} is a phenyl acetic acid, a non steroidal anti-inflammatory drug (NSAID) and is a potent inhibitor of prostaglandin synthesis. For the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and acute gouty arthritis therapeutic doses of diclofenac have been proven to be equi-efficacious when compared with other commonly used NSAIDs (Brogden *et al.,* 1980; Todd, and Sorkin, 1988). Diclofenac exhibited potent analgesic effects and is used clinically for the short term alleviation of post-operative pain, dysmenorrhoea and in various ocular conditions (Goa and Chrisp, 1992). Presenting diclofenac diethylamine transdermal dosage form is of clinical importance. Previously, we explore the effect of several penetration enhancers on the delivery of this drug through skin (Shah *et al.,* 2003). The aim of present study was to evaluate the effect of various concentrations of DMSO as enhancer on the transdermal absorption of diclofenac diethylamine.

MATERIALS AND METHODS

Materials

Diclofenac diethylamine was supplied by Novartis (China origin) and DMSO (E. Merck) was purchased from MS traders, Multan. Double distilled water from an electrically heated still, having the pH 6.8 ± 0.01 was used throughout the study. Methanol (E. Merck) and Sodium chloride (E. Merck) were used in this study. The U.V. Spectrophotometer (Agilent, Germany) was used for the analysis of the sample taken using software version-2005. Modified Franz diffusion cell (HEJ Glass Apparatus Repairing Workshop, Karachi) were used for the permeation experiments.

Animal skin

To study transdermal absorption, rabbit excised skin; in many cases full thickness is used (Hadgraft *et al.,* 2003). The dorsal full thickness skin of male rabbit (white, n=5, weighing 1-2 kg) was used as a permeation membrane. The animal skin was prepared according to standard procedures (Durrheim *et al.,* 1980; Cordero *et al.,* 1997) as described previously (Shah *et al.,* 2003).

Control solution

Ten (10) mg of diclofenac diethylamine was dissolved in 5 ml methanol and the volume was made up to 100 ml with normal saline. This solution was used as control solution without any enhancers.

Test solution

Test solutions were prepared by dissolving 1 gram of diclofenac diethylamine in 5 ml methanol and made up 100 ml with previously constituted solutions of 0.1, 0.2, 0.3, 0.4, 0.5 and 1% of DMSO in normal saline.

Diffusion cell study

Franz Diffusion cell study has been done following the same method used as described earlier in our study (*Shah et al.,* 2003).

Determination of various permeation parameters

The lag time, flux, diffustion coefficient, permeability coefficient, permeability enhancing ratio and partition coefficient were calculated using standard procedures (Badar, 1992; Tsai *et al.,* 1999; Rautio *et al.,* 2000; Shah *et al.,* 2006) given in our previous paper (Shah *et al.,* 2003).

RESULTS AND DISCUSSION

The permeation data were subjected to Microsoft Excel 2003 for analysis. Typical results have been shown in the Table I which shows diffusion co-efficient (D), initial flux value (J), permeability co-efficient (P) and enhancement ratio (ER) of the drug in control solution as well as in different concentrations of DMSO. For flux and rate constant, an increasing trend was noted and the flux was highest with DMSO at concentration of 1%.

Correlations of skin permeability coefficients to the physical properties of a wide variety of permeants have shown that skin can be effectively modeled as a simple lipid barrier to compounds having at least moderate water and oil solubilities (Potts and Guy, 1992; Kasting *et al.,* 1992; Johnson, 1997). The stratum corneum provides the skin's primary diffusion barrier (Scheuplein

Table I: Penetration kinetics of diclofenac diethylamine in control and different concentrations of DMSO

Test Solutions (%DMSO)	**Permeation Coefficient (P)** ($cm.h^{1}$)$\times10^{-4}$	**Flux (J)** ($\mu g.cm^{-2}.h^{-1}$) $\times10^{-3}$	**Diffusion Coefficient (D)** ($cm^2.h^{-1}$) $\times10^{-4}$	**Rate Constant** 0-24 h	**Enhancement Ratio (ER)**
0.1	3.34	4.6	32.98	0.0389	1.931
0.2	4.78	5.6	28.03	0.20	2.763
0.3	1.90	33.7	112.13	0.770	1.098
0.4	8.65	81.8	50.96	0.900	5.000
0.5	5.59	161.6	32.98	1.0789	3.231
1.0	3.28	242.6	19.32	1.105	1.896
Control Solution	1.73	3.7	11.87	0.0241	-

and Blank, 1971). The permeability coefficient and flux rate calculated for diclofenac diethylamine under the influence of different concentrations of DMSO showed better enhancing characteristics through hairless rabbit skin.

DMSO showed the short lag time (10 minutes to 2 hours) indicating it has a rapid enhancing effect as compared to the samples (control solution) without DMSO. The previous absorption and distribution studies on radioactivity-labelled-DMSO indicated that after a cutaneous application, the radioactivity was appeared in blood in 10 minutes in rat and after 5 minutes in man while one hour in bones (Kolb *et al.,* 1967; Denko *et al.,* 1967). The above studies supported these present findings. Non-ionized active drug molecules with low molecular weight like diclofenac are transported through the skin with DMSO while substances of high molecular weight such as insulin do not pass through the skin to significant extent. Studies have revealed that a 90% concentration of DMSO is optimal for the passage of drug dissolved in DMSO. Nevertheless the reason for an optimal effect at 90% DMSO remains unexplained (Phatek *et al.,* 1969). *In-vitro* guinea pig skin penetration studies has concluded that transdermal passage of picrate ion in the presence of DMSO follows passive diffusion which adhered to Fick's first law of diffusion. It has also been demonstrated that the absolute rate constant for the penetration of DMSO was approximately 100 times greater than that for the picrate ion. Thus, in this study diffusion co-efficient (D) of DMSO concentrations of 0.3 and 0.4% were maximum and however, the exact mechanism involved in the higher membrane penetrantion with DMSO has yet to be elucidated (Elfbaum and Laden , 1968).

Keil (1967) demonstrated that oxytetracycline satisfactorily controlled bacterial spot in peaches. Control was significantly enhanced by adding DMSO to the antibiotic spray and was applied to 0.25 and 0.5% with 66 ppm of oxytetracycline (as these concentrations were used in this study). This application gave control of the disease similar to that produced alone by 132 ppm of oxytetracycline and suggested the possibility of diluting the high-priced antibiotic with relatively inexpensive DMSO. A good evidence that 0.5% DMSO has significant carrier effects in animals is unavailable. Though the Keil's findings were attributable to a carrier effect of DMSO, but the possibility should always be considered that combining DMSO with another new compound lead to exert a greater or lesser influence on a given process or therapeutics. The influence of DMSO on the infectivity of viral nucleic acid, an indication of its transmembrane transport were evaluated and was found that DMSO enhanced polio RNA infectivity in kidney cells from monkeys. Enhancement occurred with all DMSO concentrations from 5 to 80% and was optimal at 40% with a 20-minute absorption period at room temperature. A significant percentage of nucleic acid infection was absorbed within the first 2 minutes (Amstey and Parkman, 1966).

Several studies report the increase in skin penetration of several drugs dissolved in DMSO. The increase in uterine weight after topical administration of 17-estradiol dissolved in DMSO in the immature female rat was comparable to results obtained in animals after subcutaneous administration of drug (Djan and Gunber, 1967). The percutaneous penetration of hydrocortisone and testosterone in DMSO was found to be 3 fold increase in dermal penetration (Maibach and Feldmann, 1967). Sulzberger *et al* (1967) reported that DMSO carried substances/drug rapidly and deeply into the horny layer in human and suggested the usefulness of DMSO as a vehicle for therapeutic agents in inflammatory dermatoses and superficial skin infections such as pyodermas. DMSO in some instances may carry substances such as hydrocortisone or hexachlorophene into the deeper layers of the stratum corneum, producing a reservoir which remains for 16 days and resists depletion by washing of the skin surface with soap,

water, or alcohol (Stoughton, 1965; Stoughton, 1966). As diclofenac is less soluble in water, therefore, it could be concluded that the enhanced permeation of drug may not be only due to the increased concentration of DMSO with drug into the stratum corneum, but also due to modifying the intercellular lipids, disrupting their highly ordered structure and thus increasing the permeation of the drug through the membrane. Furthermore, the latter was more important than the former in permeation because the increased amounts of drug in the skin may also be the retention of the drug by the skin. This study also verifies the idea that DMSO may offer a large and useful selection of relatively safe penetration enhancer to aid topical drug delivery.

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