

COMMUNICATION

Pharmacokinetic Profile of a New Matrix-Type Transdermal Delivery System: Diclofenac Diethyl Ammonium Patch

Kusum Devi* and K. L. K. Paranjothy

Department of Pharmaceutics, Al-Ameen College of Pharmacy,
Bangalore University, Bangalore-560 027, India

ABSTRACT

A transdermal delivery system containing the anti-inflammatory analgesic diclofenac diethyl ammonium in an ethyl hexyl acrylate and vinyl acetate pressure-sensitive adhesive system was developed for percutaneous absorption. These patches were subjected to in vitro permeation and permeation enhancement studies through rat skin using a specially designed diffusion cell. Further, the work deals with percutaneous absorption studies carried out on both animals and human volunteers. The pharmacokinetic parameters calculated from the blood levels of the drug reveal a profile typical of a sustained-release formulation, with the ability to maintain adequate plasma levels for 24 hr (i.e., up to the next application). (Area under the curve [AUC]: 4.356 ± 1.3 mcg/ml.hr in animals and 0.442 ± 0.053 mcg/ml.hr in humans; T_{max} was 8 hr in both the cases, whereas C_{max} was 0.288 ± 0.088 mcg/ml in animals and $0.034 \pm .008$ mcg/ml in human volunteers.) The amount of the drug bioavailable for targeting the sites of action is lower than via the oral route, but the absorbed dose appears to be adequate for therapeutic use, particularly because of the absence of side effects.

* To whom correspondence should be addressed.

INTRODUCTION

Transdermal drug delivery has the advantage of being both precise and consistent, like a closely monitored intravenous drip, but it is noninvasive because there is no needle involved. Diclofenac diethyl ammonium is a non-steroidal anti-inflammatory drug (NSAID) used for the symptomatic relief of pain and inflammation in patients suffering from musculoskeletal diseases. In recent years, there has been an increasing interest in the development of anti-inflammatory drugs for topical usage. It has been recorded in literature that diclofenac, when applied topically, penetrates the skin barrier in an amount sufficient to exert the desired level of local therapeutic activity (1,2).

In contrast to conventional formulations (cream, gel, ointment, etc.), the diclofenac diethyl ammonium transdermal patch permits, by occlusive bandage and slow release of the drug, a constant and continuous presence of the active ingredient at the inflamed area.

For the practical use of the newly developed diclofenac transdermal patches in human therapy, in vitro evaluation and pharmacokinetic studies needed to be performed in both animals and human volunteers.

MATERIALS AND METHODS

Diclofenac diethyl ammonium (a kind gift by BPRL, Bangalore, India); polyvinyl pyrrolidone [S.D. Fine Chemicals(P) Ltd., Bombay, India]; propylene glycol (Qualigens, Mumbai, India); chloroform, methanol, and acetonitrile of high-performance liquid chromatography (HPLC) grade (Ranbaxy Labs, New Delhi, India); and ethyl hexyl acrylate and vinyl acetate, which is an adhesive film former (3M Silicones, New York). This adhesive film former was nontoxic and nonabsorbable and did not lose its adhesive properties when formulated with the drugs and excipients. All the other chemicals used in the study were of analytic reagent grade.

Preparation of the Transdermal Patches

For preparation of the transdermal patches, 300 mg of the acrylate adhesive was dissolved in 5 ml chloroform, followed by 400 mg polyvinyl pyrrolidone, which was also dissolved in the adhesive solution by slow stirring. Then, 360 mg of the drug was added to the clear solution and stirred slowly under covered conditions until it completely dissolved. Propylene glycol (0.4 ml) and metha-

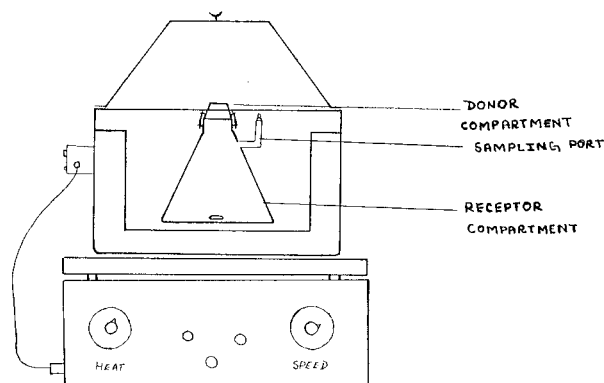
nol (1.6 ml) were finally added and stirred well to get a clear solution.

Next, 1.2 ml of the casting solution was pipetted onto the center of stainless steel rings with smooth surfaces and placed on the backing layer of pearlesced polyester so that it formed a well. The solution was dried at room temperature for 24 hr. Patches with a 5-cm diameter containing 60 mg of the drug uniformly distributed in the adhesive were cut. These patches were covered with silicone-coated paper as a covering liner and were stored for further evaluation.

In Vitro Skin Permeation and Permeation Enhancement Studies

Freshly excised albino rat abdominal skin was used as the permeation barrier. A diffusion cell was fabricated on the lines of a Keshary Chein cell (3) to carry out in vitro evaluation (Fig. 1).

The skin section and the patch were mounted carefully between the donor and receptor compartments of the diffusion cell and held securely by springs. The donor compartment was empty and open to the atmosphere, but the receptor compartment was filled with distilled water (65 ml). The temperature of the diffusion cells was main-



DIFFUSION CELL

Volume of receptor compartment	: 65 ml
Diameter of membrane (rat skin) used for study	: 3.0 cm
Diameter of the film used for study	: 2.4 cm
Temperature of the study	: 32 ± 2°C
Degree of turbulence in receptor medium	: 45 rpm

Figure 1. Diffusion cell for in vitro evaluation.

tained at 32°C by immersion in a water bath with sides that were insulated and which had a thermostat. The cells were continuously agitated. Samples were withdrawn from the receptor compartments at predetermined intervals and replaced with fresh receptor medium. The samples were analyzed spectroscopically at 450 nm after suitable dilution.

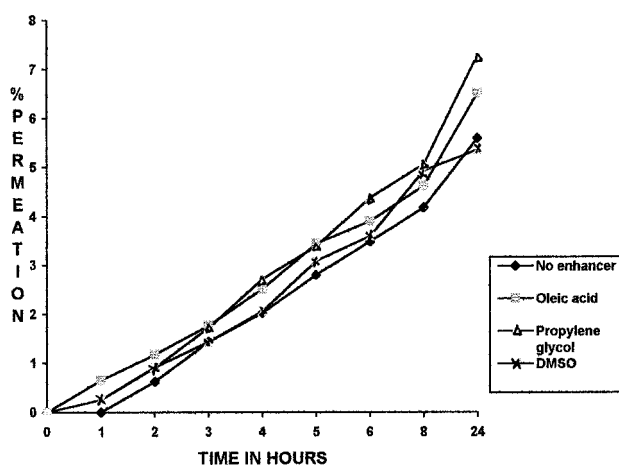
To optimize the penetration, and hence the absorption, of the drug from the transdermal patches, penetration enhancers were used. They included oleic acid, propylene glycol, dimethyl sulfoxide, hexane, and ethanol. These enhancers were used in two different ways: (a) by inclusion into the patches at a concentration of 5% w/v (4) and (b) by treating the freshly excised rat abdominal skin on the stratum corneum side with 2 ml of the enhancer individually or with a combination of enhancers (5).

Results obtained are presented graphically in Graph 1 and Graph 2.

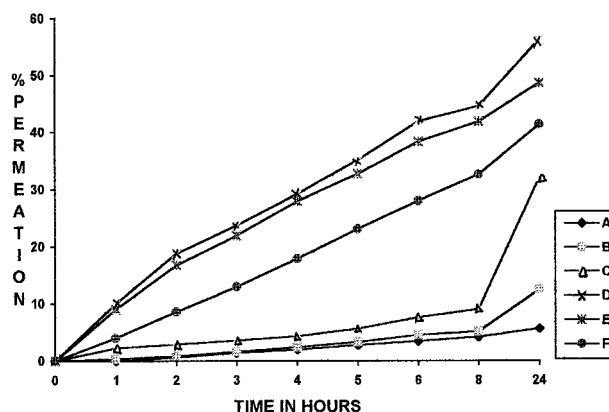
Pharmacokinetic Evaluation of the Patches in Animals

Rabbits have been used as models for bioavailability studies among the various animal models. The experiment was conducted on six rabbits weighing between 1.8 and 2.0 kg. Before commencement of the experiment, the skin of every rabbit was thoroughly examined for any abnormality, and only those having no structural abnormality of the skin were included.

A skin area of around 50 cm² was shaved, covering both sides of the vertebral column of each rabbit, and



Graph 1. Effect of penetration enhancers on the in vitro permeation of diclofenac diethyl ammonium.



Graph 2. Effect of solvent treatment and its duration on the in vitro permeation of diclofenac diethyl ammonium. A, no enhancer; B, DMSO (1 hr); C, ethanol + hexane (1 hr); D, ethanol + hexane + propylene glycol (1 hr); E, ethanol + hexane + propylene glycol (1/2 hr); F, ethanol + hexane + propylene glycol (10 min).

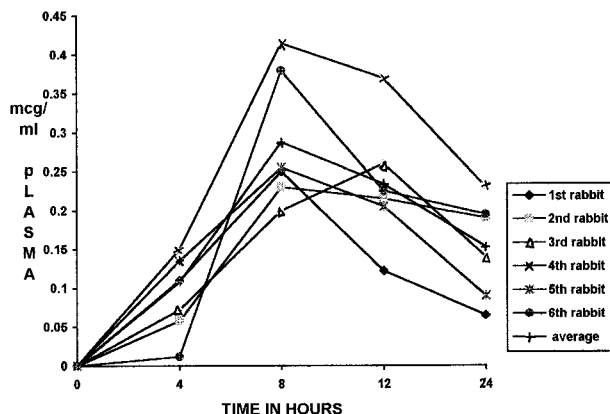
care was taken to avoid damage to skin during shaving. Before application of the patches, rabbits were kept for 24 hr under observation for any untoward effect of shaving to the skin and were fasted over this period. The patches containing 60 mg of diclofenac diethyl ammonium were applied on the shaved surface.

Next, 2 ml blood was withdrawn from the ear vein of each rabbit at 0, 4, 8, 12, and 24 hr intervals into heparinized tubes to prevent the coagulation of blood. Due to the small size of the animal and damage to the ear vein, it was not possible to withdraw the blood for more than the above-mentioned intervals (6).

Blood samples were centrifuged for 30 min at 5000 rpm, and the plasma was separated and kept in the refrigerator until analysis by the standardized HPLC procedure. The blood levels of the drugs obtained are recorded in Graph 3.

Pharmacokinetic Evaluation of the Patches in Human Volunteers

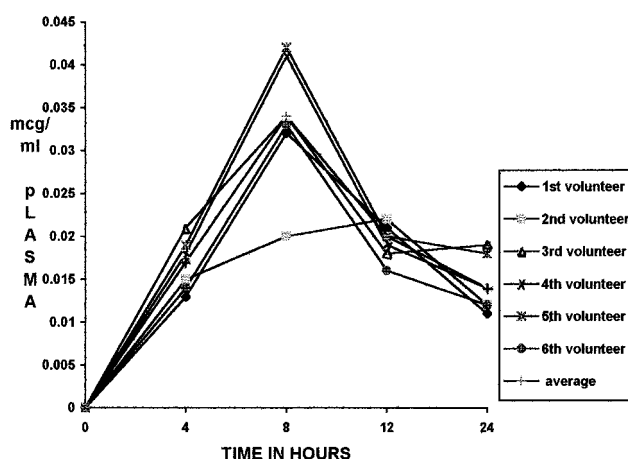
The study was conducted in six healthy male volunteers, aged between 21 and 23 years, and weighing about 50–60 kg. Drug administration and blood withdrawal were carried out under the direct supervision of a doctor and with the assistance of a trained medical laboratory technician. The volunteers were asked to abstain from any drug or alcohol for 1 week and were deprived of food for 12 hr prior to the study.



Graph 3. Blood levels of diclofenac diethyl ammonium from transdermal patches in rabbits.

The transdermal patches containing 60 mg of diclofenac were applied on the anterior surface of the forearm, near the elbow. The volunteers were instructed not to remove the patch and also to observe for any sign of irritation at the site of application. Blood samples (10 ml) were collected with the help of a syringe, which was rinsed with a dilution of heparin into heparinized tubes at time intervals of 0, 4, 8, 12, and 24 hr after application of the patch. The plasma was immediately separated by centrifugation for 30 min at 5000 rpm and stored in the refrigerator for further analysis.

The drug in the plasma was analyzed by HPLC, and the blood levels obtained are recorded in Graph 4.



Graph 4. Blood levels of diclofenac diethyl ammonium from transdermal patches in human volunteers.

High-Pressure Liquid Chromatographic Analysis

The animal and human blood samples were analyzed for drug content by an HPLC method (7). A high-performance liquid chromatograph equipped with a SPD-10A UV-Vis detector, LC-10AD pump, and a C-R7A Plus Integrator from Shimadzu-Japan was used for the drug analyses. A C18 column was used. The plasma proteins were precipitated with 5 M hydrochloric acid. A pinch of sodium chloride was added to each tube to prevent emulsification during the extraction process. The sample was vortexed with 5 ml ethyl acetate for 5 min. The organic layer was separated by centrifugation at 2000 rpm for 10 min and dried in vacuum. The residue was reconstituted with the mobile phase (0.1 ml) and injected (20 μ l) into the column. The analysis was carried out at a wavelength of 274 nm. A mixture of methanol, acetonitrile, and water (40:40:25) was used as the mobile phase at a flow rate of 1.5 ml per min. The concentrations of samples were calculated from a standard graph (200 ng/ml–1000 ng/ml concentration range) prepared from peak area versus concentrations of diclofenac ($r = .990$).

Statistical Analysis

All the in vitro data are reported as mean \pm SD of the mean. The in vivo data (i.e., animal and human blood levels) were tested for statistical significance using analysis of variance (ANOVA) and were found to be significant on the basis of the F value ($p < .01$).

Serial No.	Study	F value
1	Diclofenac diethyl ammonium in animals	$F_{(3,20)} 7.900$
2	Diclofenac diethyl ammonium in humans	$F_{(3,20)} 20.756$

Adverse Reactions

The animals and the human volunteers were kept under close observation for any adverse effect that occurred during the study, which were reported as to the time of onset, nature, duration, and severity.

RESULTS

The in vitro mean cumulative percentage permeation versus time profiles of diclofenac were linear, which indicates zero-order kinetics of drug permeation (Graph 1). It is also clear from Graph 1 that the amount of drug that

Table 1
Mean Pharmacokinetic Parameters of Diclofenac Diethyl Ammonium

Sl. No.	Parameters	Mean \pm SD in Rabbits	Mean \pm SD in Humans
1	C_{max} (mcg/ml)	0.288 \pm 0.088	0.034 \pm 0.008
2	T_{max} (hours)	8.000 \pm 0.000	8.000 \pm 0.000
3	K_e (hour ⁻¹)	0.144 \pm 0.020	0.070 \pm 0.006
4	$t_{1/2}$ (hr)	4.893 \pm 0.800	9.966 \pm 0.834
5	AUC (0 \rightarrow 24) mcg/ml.hr	4.356 \pm 1.300	0.442 \pm 0.053
6	AUC (0 \rightarrow ∞) mcg/ml.hr	5.663 \pm 1.656	0.637 \pm 0.084
7	MRT (hr)	11.462 \pm 2.409	15.388 \pm 0.426

permeated through the rat skin from the plain patch (no enhancement technique employed) was rather low; hence, permeation-enhancement techniques have to be employed to enhance the permeation. Although a moderate increase in permeation of the drug was brought about by incorporating the enhancers in the patch, a definite increase in the permeation was exhibited on treating the skin on the stratum corneum side with a solvent mixture. The maximum enhancement was around 7.5-fold, brought about by a hexane/ethanol/propylene glycol solvent mixture; this increase in permeation was proportional to the duration of solvent treatment.

From the percutaneous absorption studies, various pharmacokinetic parameters (8) of the drug were calculated (Table 1). Excellent correlation was observed between the animal and human volunteer studies. Measurable concentrations of diclofenac diethyl ammonium were obtained in the plasma from the fourth hour in animals and also in human volunteers. The concentration of the drug reached the maximum at the eighth hour; a C_{max} of 0.034 μ g/ml was obtained for humans, and a C_{max} of 0.288 μ g/ml was obtained for animals (rabbits). Thereafter, the concentration of the drug reduced gradually, but even at the 24th hour, measurable quantities of diclofenac persisted in both animals and humans.

The area-under-the-curve (AUC) values (which more correctly define bioavailability), calculated using the trapezoidal rule, indicated an extent of drug availability of 4.356 μ g/ml.hr of diclofenac from the transdermal dosage form in rabbits compared to 0.442 μ g/ml.hr in the human volunteers.

The elimination rate constant K_e calculated from the linear terminal elimination phase was found to be 0.144/hr for diclofenac in rabbits compared to 0.070/hr in humans.

The calculated parameters also indicate that the biological half-life of diclofenac is prolonged from 2 hr (conventional tablets) to 4.9 hr in animals and to 9.9 hr in human volunteers; hence, the drug administered in the transdermal dosage form will remain in the body for a longer period and thus will exert a sustained action.

DISCUSSION

Regression analysis of the in vitro (Graphs 1 and 2) permeation curves was carried out, and the slope of the straight line was obtained after plotting; the mean cumulative amount permeated per patch (μ g/mg) versus time was taken as the experimental skin flux for diclofenac diethyl ammonium. The cumulative mean flux values of diclofenac from the patch, which was subjected to in vitro permeation studies after treating the rat skin with the hexane/ethanol/propylene glycol solvent system, was 54.78 μ g/cm²/hr was significantly higher than that obtained with the plain patch, which was 6.88 μ g/cm²/hr.

Thus, it can be concluded that the drug diclofenac permeates the skin when administered transdermally and attains measurable concentrations in the bloodstream within 4 hr. Since this dosage form can be administered at the site of action itself, the drug not only permeates the skin and reaches the systemic circulation, but also concentrates itself at the site of action; hence, it is possible to achieve maximum pharmacological effects with minimum quantities of the drug, thus making it possible to reduce the dose administered through this route with a concomitant reduction in side effects associated with the drug.

The patches did not show any signs of erythema, edema, necrosis, ulceration, or pigmentation in both ani-

mal and human volunteer studies. The above safety data obtained indicate that the transdermal patch of diclofenac diethyl ammonium has a very good local tolerability in both animals and humans, while there is no need to deal with possible adverse systemic effects.

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