DRUG DEVELOPMENT RESEARCH 58:283–290 (2003)

DDR

Research Article

Influence of Vehicles on the In Vitro Percutaneous Absorption of Piroxicam to Optimise the Formulation of Patch Tests in Dermatology

Stéphanie d'Arpino,[1,2] Valérie Corbrion-Archer,[1] Jean-Paul Marty,[2] Laurent Lantieri,[3] Claire-Marie Vincent,[2] Alain Astier,[1] and Muriel Paul[1]*

[1]*Pharmacy Department, Henri Mondor Hospital, Créteil, France*

[2]*Faculty of Pharmacy, University of Paris South, Dermopharmacology and Cosmetology Research, Châtenay-Malabry, France*

[3]*Plastic Surgery Department, Henri Mondor Hospital, Créteil, France*

Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The present study evaluated the influence of various vehicles on the in vitro percutaneous absorption of piroxicam across human skin under standard diffusion conditions to optimise the formulation of patch tests in dermatology. The transdermal permeation properties of piroxicam were evaluated from five vehicles (PEG 400, propylene glycol, transcutol P, petrolatum, and petrolatum + transcutol P 10%) using Franz diffusion cells. Piroxicam was applied as a saturated solution in vehicles to ensure equal thermodynamic activity of piroxicam in the vehicles. Concentrations of piroxicam were determined by high performance liquid chromatography in epidermis, dermis, and receptor liquid. The release of piroxicam from its vehicles and consequently its cutaneous absorption are influenced by the vehicle assayed and the solubility of piroxicam in each vehicle. The penetration of piroxicam through human skin was clearly enhanced by propylene glycol and petrolatum + transcutol P 10%, with cumulated amounts of piroxicam in the receptor fluid at 48 h of 116 ± 32 and 159 ± 28 $\mu g/mg/cm^2$ respectively, whereas petrolatum alone did not allow the diffusion of piroxicam. Additional experiments should be conducted in vivo to correlate the results obtained in vitro and to provide clinicians with a more reliable diagnostic test. Drug Dev. Res. 58:283–290, 2003.

Key words: Franz-type diffusion cell; in vitro percutaneous absorption; patch tests; piroxicam; vehicles

INTRODUCTION

Nonsteroidal antiinflammatory drugs are widely used as analgesics, and in the treatment of locomotor pathologies and of local inflammation. For the treatment of inflammatory diseases and pain, piroxicam is available on the market under systemic or topical formulations. These therapies are very effective [Carrabba, 1995; Abe, 1982], but the use is often limited because of their potential to cause adverse effects [Gerber, 1987]. The predominant site of adverse effects is the gastrointestinal system [Phelip, 1984; Pitts,1982], but several adverse skin reactions have been reported [Stern and Bigby, 1984; Vasconcelos et al., 1997; Katoh, 1995; Guillaume, 1985]. They can occur in 1% to 7% of patients taking piroxicam [Pitts, 1982]. To identify the etiological drug, a reliable diagnosis is essential but often difficult. Patients are

*Correspondence to: Muriel Paul, Henri Mondor Hospital, 51, av. du Maréchal de Lattre de Tassigny, 94010 Créteil Cedex, France.
E-mail: muriel.paul@hmn.ap-hop-paris.fr

Received 1 January 2003; Accepted 22 January 2003

Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/ddr.10164

frequently taking multiple medications, and it is not possible to determine the offending agent on the basis of the clinical picture. Oral provocation is the gold standard for diagnosis, but it is not always feasible. As a result, physicians have searched for a valid diagnostic test that does not have the inherent dangers of oral challenge. The patch test is a safe alternative and can be a real diagnostic tool if conducted and controlled under specific circumstances.

Patch testing is an established method of diagnosing cutaneous reactions due to piroxicam [Barbaud et al., 1998]. Although safe and easy to perform, patch tests have not been shown to be particularly accurate. This can be explained by the lack of guidelines concerning the procedure to use for each drug, such as the drug concentration and the vehicle. Authors have suggested formulating the suspected drug at a concentration of 10% in white petrolatum [Milpied-Homsi, 1995; Alanko et al., 1987]. However, many patch tests containing piroxicam in formulation with petrolatum provided negative results, certainly due to the low affinity between the two products [Katoh et al., 1995; Barbaud et al., 1998].

The objective of this study was to analyse the influence of different vehicles on the in vitro transdermal absorption of piroxicam and to optimise the formulation of patch tests in order to give to clinicians a selective, sensitive, and reliable patch test.

In the present study, we compare the transdermal permeation properties of piroxicam from various vehicles (PEG 400, propylene glycol, transcutol P, petrolatum, and petrolatum + transcutol P 10%) across human skin in vitro.

MATERIALS AND METHODS

Chemicals and Reagents

Piroxicam was supplied by Sigma (St. Louis, MO, U.S.A.). PEG 400, propylene glycol, paraffin oil, and petrolatum were provided by Cooper (Melun, France) and transcutol P by Gattefossé (St. Priest, France).

Acetonitrile and methanol were from Carlo Erba (Val de Reuil, France), chlorhydric acid and sodium hydroxide from Merck (Darmstadt, Germany), orthophosphoric acid and ethanol from Prolabo (Fontenay sous Bois, France), and finally phosphate buffer solution (pH 7.4, 10 mmol/L) from Sigma Diagnostics (St. Louis, MO, U.S.A.).

All reagents used were of analytical grade.

Determination of Piroxicam Solubility in Each Vehicle

To ensure an equal and maximal thermodynamic activity, the concentration of piroxicam was determined at saturation in each formulation. An excess of piroxicam was added to the various vehicles (PEG 400, propylene glycol, transcutol P, paraffin oil). The products were mixed for 24 h with a horizontal stirrer Speci-Mix (Bioblock Scientifics, Strasbourg, France). After separation by centrifugation at 3,000 rpm (Jouan BB, St. Herblain, France), the supernatant was diluted in methanol, then the solution was injected into a high-performance liquid chromatography (HPLC), which is described below.

A first step was needed for the determination of the saturated concentration of piroxicam in petrolatum and petrolatum + transcutol P 10%. It was studied in paraffin oil, which allowed a better homogeneity of the formulation.

Determination of Piroxicam Stability in NaOH 1 N

A stability study of piroxicam was lead in NaOH 1 N to test whether piroxicam could resist a chemical treatment.

A solution of piroxicam in NaOH 1 N was prepared at concentration of 50 μg piroxicam/mL. One milliliter of this solution was neutralised by 100 μL HCl 10 N. After dilution in methanol, the piroxicam ultraviolet (UV) spectrum was compared with a UV spectrum from a methanolic solution and measurements were made with HPLC.

The stability of piroxicam solution was assayed during 24, 48, and 117 h at 60°C. Each experiment was carried out in triplicate.

Study of the Percutaneous Diffusion In Vitro

Preparation of diffusion cells

Percutaneous absorption was measured in vitro across human skin following the guidelines published by the AAPS and the FDA [Skelly et al., 1987]. Franz glass diffusion cells were used. The area available for diffusion was 1.76 cm^2 and the volume of the receiver or dermal compartment was approximately 6 mL. The liquid in the receptor compartment was a phosphate buffer solution (pH 7.4, 10 mmol/L).

Abdominal human skin was obtained from plastic surgery (Henri Mondor Hospital, Créteil, France) and was frozen until ready to use. Three skin biopsies, from two white female donors (39 and 53 years old) and one female African donor (32 years old), were dermatomed to a constant thickness of 400 μm with a Brown Dermatome (Zimmer, Vitry sur Seine, France). The dermatomed human skin was cut, with surgical scissors, into squares approximately 2 cm^2, but large enough to cover the opening of the diffusion cell. Using forceps, the skin was placed on the top side of the dermal compartment, the dermis facing the dermal compartment of the cell. The top of the cell was then clamped

over the bottom of the diffusion cell to hold the skin in place, with the epidermal side up. Each cell was filled with the receiver liquid using a polyethylene syringe and then was gently inverted to release all air bubbles in order to ensure good contact between the dermal side of the biopsy and the receiver liquid. The dermal compartment of the cells was maintained at 37°C with a surrounding water circulation. A magnetic stirrer was used to obtain chemical and temperature homogeneity of the receiver liquid in the dermal compartment. Stirring started immediately once the entire filling operation was terminated. Prior to the application of the formulation on the epidermal surface, the cells were allowed to equilibrate with the atmosphere of the laboratory for 2 h.

Application of piroxicam formulations

Each diffusion cell was attributed to one of the tested formulations. About 50 mg of each formulation exactly individually weighed was applied with a micropipette of 50 µL (Nichiryo Co., Ltd., Japan) (for liquid vehicles) or with a glass microspatula (for viscous vehicles) on the well-defined surface of the epidermis. Each cell was then kept open to the air and "climatic" conditions of the laboratory (24°C). These nonoccluded conditions allow evaporation of the volatile components of the formulations as in a clinical situation.

Sample collection

During the experiment, the receptor fluid was completely collected and replaced by approximately 6 mL of fresh medium, at 0, 3, 6, 7.5, 24, 32, and 48 h after the application. At the end of the experiment, the surface of each biopsy was washed twice with 500 µL of dichloromethane applied gently with a pipette. Then the diffusion cells were dismantled and the epidermis was separated from the dermis with a forceps. The piroxicam concentrations in the receiver samples were measured by HPLC.

Treatment of epidermis and dermis samples

In a glass tube containing the epidermis sample, 1 mL of NaOH 1 N solution was added. After incubation in a waterbath of 50°C for 48 h, the mixture was neutralised with 100 µl of HCl 10 N, then diluted with 1.1 mL of methanol. This suspension was vortex-mixed and then filtered. The concentration of piroxicam in the filtered solution was determined by HPLC.

In a glass tube containing the dermis sample, 2 mL of NaOH 1 N solution was added. After incubation in a waterbath of 50°C for 48 h, the mixture was neutralised with 200 µL of HCl 10 N, then diluted with 2.2 mL of methanol. This suspension was vortex-mixed and then filtered. The concentration of piroxicam in the filtered solution was determined by HPLC.

Quality control of the percutaneous diffusion method

A double quality control was performed on each piece of skin from each diffusion cell. The skin thickness (expressed in micrometers) was measured with a caliper-square (G. Boutillon, Chenôve, France) before being placed on the diffusion cell. Then, the integrity of the skin was checked prior to the application of the formulation by measuring the transepidermal water loss (TEWL in $g/m^2 \cdot h$) with a Tewameter TM 210 (Courage & Khazaka, Cologne, Germany). The TEWL measurements were performed according to the following procedure: the probe was applied on the top of the epidermal side of the diffusion cell, and TEWL was measured during 2 min in order to obtain a state of equilibrium. Then, during the next 60 sec, the TEWL was registered. Skin thickness and TEWL measurements were determined for the 30 diffusion cells.

Chromatographic Conditions

The amounts of piroxicam in the epidermis, dermis, and receptor fluid were quantitated by HPLC with a C18 column (Spherisorb ODS-2, 5 µm, Waters, St. Quentin en Yvelines, France) mounted with a precolumn (Spherisorb ODS-2, Waters). Measurements were made with a Jasco PU-980 pump (Jasco, Nantes, France) equipped with an automate injector Jasco AS-1555-10 (Jasco). UV detection was effected at 330 nm with a Jasco UV-975 (Jasco). The mobile phase, which consisted of acetonitrile-orthophosphoric acid-distilled water (50/5/45, vol/vol), was pumped at flow rate of 1 mL/min. The chromatograms were integrated using a C-R6A Chromatopac (Shimadzu, Kyoto, Japan). The chromatographic method was based on the study of Twomey et al., and the validation of the analytical methods was previously done in the laboratory [Twomey et al., 1980].

Data Analysis

For each formulation, percutaneous penetration of piroxicam was evaluated from the following permeation parameters: permeability rate constant K_p, lag time T_{lag}, diffusion coefficient D_m, and vehicle/stratum corneum partition coefficient K_m.

According to Fick's law of diffusion [Kim et al., 1992; Bach and Lippold; 1998], the cumulative amount (Q) of drug appearing in the receptor fluid as a function of time (t) (dQ/dt in µg/h) is expressed as

$$(dQ/dt) = K_p \cdot S \cdot (C_0 - C_1), \quad (1)$$

where K_p is the permeability rate constant (μm/h), S is the effective diffusion area (cm^2), C_0 is the drug concentration in the formulation in the donor compartment (μg/mL), and C_1 is the drug concentration in the receptor fluid (μg/mL).

As S represents the surface of Franz diffusion cells and therefore is constant, the permeability rate constant K_p was determined from the slope of the steady-state portion of the graph $(dQ/dt) = K_p \cdot (C_0 - C_1)$.

The lag time values T_{lag} were obtained at steady state from the *x*-intercept of the linear portion of the cumulative amount (micrograms) of the drug permeated versus time (h).

The diffusion coefficient D_m is defined from the lag time T_{lag} according to Eq. 2 [Bach and Lippold, 1998]:

$$D_m = e^2/(6 \times T_{lag}), \quad (2)$$

where e denotes the thickness of the skin (micrometers).

The partition coefficient K_m of the drug between the stratum corneum and the vehicle was calculated from the following equation [Kim, 1992; Bach and Lippold; 1998] :

$$K_m = (K_p \times e)/D_m, \quad (3)$$

where k_p, e, and D_m are known.

For each formulation, an exposure index (E.I.) was calculated at the junction of epidermis/dermis from Eq. 4:

$$E.I. = [(Q_D + Q_{RF})/T_{exp}] \times (T_{exp} - T_{lag}), \quad (4)$$

where Q_D is the cumulative amount of drug in the dermis, Q_{RF} is the cumulative amount of drug appearing in the receptor fluid, T_{exp} is the duration of the diffusion experiment, and T_{lag} the lag time.

Data Expression

The amounts of piroxicam detected in the cutaneous compartments and in the receptor phase from the different formulations during 48 h of diffusion are expressed in micrograms per milligram per square centimeter, i.e., the cumulative amount of piroxicam detected in one compartment (micrograms) divided by the amount of piroxicam applied (milligrams) and divided by the area available for diffusion (square centimeter). This takes into consideration only the quantity of piroxicam that diffused across the skin.

The mass balance expressed in % has been achieved for each formulation by division of the quantity of piroxicam (micrograms) measured in each compartment by the amount of piroxicam applied (micrograms).

Statistical Methods

For each group of diffusion cells, the basic statistics were concerning the calculation of the mean and of the associated standard deviation. The data analyses are expressed as mean values ± standard error. In most cases, six diffusion cells per formulation were tested.

RESULTS

Solubility of Piroxicam in Each Vehicle

Piroxicam showed a higher saturated solubility in PEG 400 and Transcutol P, with solubility values of 23.1 and 13.9 mg/mL, respectively. An intermediate solubility of 1.41 mg/mL was found in propylene glycol and petrolatum + transcutol P 10%. Piroxicam showed a very low solubility in petrolatum (0.024 mg/g).

Stability Test in NaOH 1 N

After 5 days in NaOH 1 N at 60°C, piroxicam did not show any degradation. The chemical stability of piroxicam in NaOH is good enough to allow the in vitro experiment.

Quality Control of the Diffusion Method

The mean thickness of the skin was 419 ± 66 μm. The mean TEWL was 5.23 ± 1.08 $g/m^2 \cdot h$.

In Vitro Percutaneous Diffusion

The cumulative amounts of piroxicam detected in the receptor phase from the different formulations during 48 h of diffusion are presented in Figure 1.

At the end of the 48 h of measurement, diffusion profiles achieved using Franz diffusion cells showed no diffusion of piroxicam in the receptor fluid for the formulation containing petrolatum and a low diffusion for PEG 400 and transcutol P, with amounts of about 5 $\mu g/mg/cm^2$. The diffusion of piroxicam across the skin from the formulations petrolatum + transcutol P 10% and propylene glycol was higher than for the other formulations, with a faster and more intensive diffusion of piroxicam in formulation petrolatum + transcutol P 10% than in propylene glycol (159 ± 28 $\mu g/mg/cm^2$ versus 116 ± 32 $\mu g/mg/cm^2$).

As shown in Figure 2, the distribution of piroxicam was not homogeneous in the cutaneous compartments (epidermis, dermis) and in the receptor fluid. In the dermis, the amount of piroxicam permeated was low whatever the vehicle tested, with values lower than 27 $\mu g/mg/cm^2$. In the epidermis, the highest amounts of piroxicam were observed with propylene glycol (134 ± 47 $\mu g/mg/cm^2$), petrolatum (74 ± 37 $\mu g/mg/cm^2$), and petrolatum + transcutol P 10% (67 ± 16 $\mu g/mg/cm^2$). Petrolatum + transcutol P

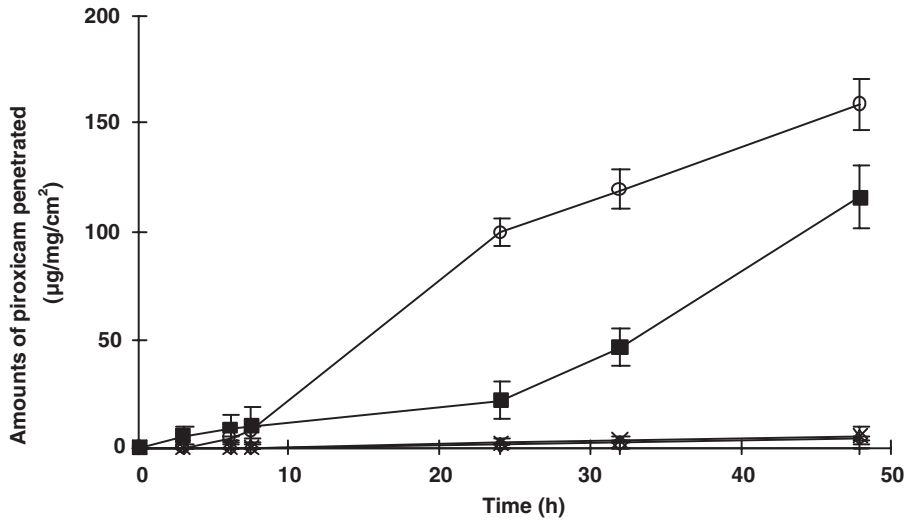

Fig. 1. Evolution of cumulative amounts of piroxicam in the receptor fluid in formulation with PEG 400 (×), propylene glycol (■), transcutol P (◆), petrolatum (+), and petrolatum + Transcutol P 10% (○).

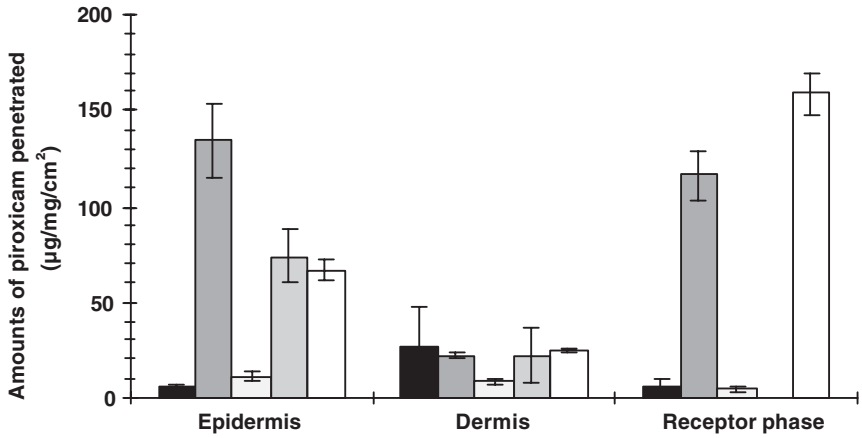

Fig. 2. Distribution of piroxicam in the cutaneous compartments (epidermis, dermis) and in the receptor phase after application of formulations for 48 h on human skin in vitro. Each column shows the mean of six experiments ± standard error. Each bar is represented as follows: ■ PEG 400, ■ propylene glycol, ▨ Transcutol P, ▨ petrolatum, and □ petrolatum + Transcutol P 10%.

10% and propylene glycol allowed piroxicam to permeate in large amounts to the receptor fluid, with values of 159 ± 28 and 116 ± 32 µg/mg/cm^2, respectively.

The mass balance was achieved for all formulations except for petrolatum. Its high viscosity did not allow measurement of the quantities of piroxicam in the samples. For the four other formulations, this balance consisted of between 82% and 111% of the applied dose of piroxicam.

Data Analysis

Lag time (T_{lag}), vehicle/stratum corneum partition coefficient (K_m), diffusion coefficient (D_m), and permeability rate constant (K_p) are summarised in Table 1. These parameters could not be calculated for petrolatum because the diffusion did not reach the receptor phase. The vehicle that provided the highest K_p value was petrolatum + transcutol P 10% (0.00239 ± 0.000017 µm/h); then K_p gradually decreased with PEG 400 (0.00117 ± 0.00012 µm/h), transcutol P (0.000993 ± 0.00008 µm/h), and propylene glycol (0.000 976 ± 0.000065 µm/h). The increases in K_p were paralleled by shortened lag times for all formulations, with the shortest T_{lag} for petrolatum + transcutol P 10% (3.02 ± 0.46 h). Even if K_m values were high with propylene glycol (0.000258 ± 0.000028), PEG 400 (0.000216 ± 0.000047), and transcutol P

TABLE 1. Values of the Permeation Parameters of Piroxicam Obtained After 48 H of Diffusion Through Human Skin In Vitro

Vehicle	T_{lag}^a (h)	K_m^a	D_m^a ($\mu m^2/h$)	K_p^a ($\mu m/h$)
Propylene glycol	19.3±2.08	0.000258±0.000028	1.619.8±291.3	0.000976±0.000065
10% transcutol in petrolatum	3.02±0.46	0.000115±0,000014	8.532.8±1798.2	0.00239±0.000017
PEG 400	13.7±2.80	0.000216±0.000047	2.998.7±829.2	0.00117±0.00012
Transcutol	15.1±1.23	0.000200±0.000023	1.962.0±153.0	0.000913±0.00008
Petrolatum	—	—	—	—

[a]T_{lag}, lag time;
K_m, vehicle/stratum corneum partition coefficient;
D_m, diffusion coefficient;
K_p, permeability rate constant. Results are expressed as mean±standard error.

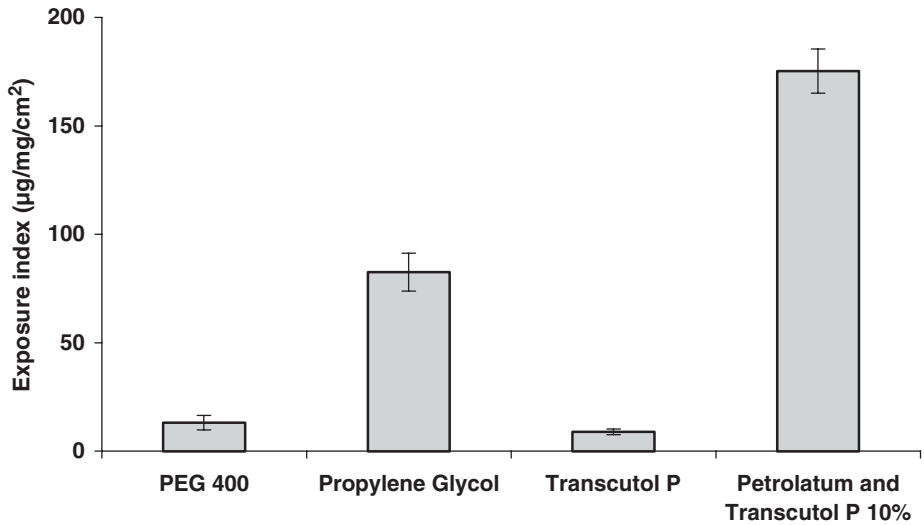

Fig. 3. Values of exposure index of piroxicam at the junction of epidermis/dermis compartments after application of formulations for 48 h on human skin in vitro.

(0.00020±0.000023), D_m still remained low with values between 1,600 and 3,000 $\mu m^2/h$. The vehicle petrolatum + transcutol P 10% provided the highest value D_m (8,532.8±1,798.2 $\mu m^2/h$).

The E.I. were calculated for the different formulations, except for petrolatum that did not allow piroxicam to diffuse through the skin. The values are presented in Figure 3. The highest exposure index of piroxicam was found when the vehicle used was petrolatum + transcutol P 10%, with an exposure index of 175.3±10.18 $\mu g/mg/cm^2$. Then the exposure index gradually decreased when piroxicam was formulated in propylene glycol (82.5±8.75 $\mu g/mg/cm^2$), PEG 400 (13.1±3.30 $\mu g/mg/cm^2$) and transcutol P (8.92±1.3 $\mu g/mg/cm^2$).

DISCUSSION

The release of piroxicam, a lipophilic drug, and therefore its percutaneous absorption were remarkably affected by the type of vehicle used in the formulation and by the solubility of piroxicam in the various vehicles.

Actually, only the influence of vehicle was variable. Indeed, piroxicam concentrations reached saturation in the five vehicles tested, and their thermodynamic activities were equal among the vehicles. The skin thickness was homogeneous and corresponding to our internal specifications. The measurement of TEWL on each biopsy before the application of the product under investigation was responding to the specific objective to assess that the skin integrity and the experimental setting were good.

A remarkable diffusion was found when the drug was applied with propylene glycol and petrolatum + transcutol P 10%, with cumulative amounts of piroxicam at 48 h in the receptor phase of 116±32 and 159±28 $\mu g/mg/cm^2$, respectively (Fig. 1). At 48 h, the

amounts of piroxicam in all compartments were 3- to 10-fold greater as compared to the others. From the solubility test, the solubility of piroxicam at saturation was 1.41 mg/mL in both vehicles, propylene glycol and petrolatum + transcutol P 10%. Thus, the high percutaneous absorption of piroxicam from these vehicles may be due to its moderate solubility. These results are in agreement with the study of Okamoto et al. on percutaneous absorption of acyclovir [Okamoto et al., 1990]. They showed that solubility parameters determined the penetration rate of acyclovir. However, although propylene glycol is known to act as a penetration enhancer of piroxicam [Okuyama et al., 1999; Santoyo et al., 1996], the permeation profiles showed a higher and faster diffusion of piroxicam to the receptor fluid from the combination petrolatum + transcutol P 10% (Fig. 1). With the use of this vehicle, the value of the exposure index of piroxicam was twofold greater than that of propylene glycol (Fig. 3). As shown in Table 1, the D_m value (diffusion coefficient) of piroxicam representing its diffusion was approximately fivefold greater for petrolatum + transcutol P 10% than for propylene glycol ($8{,}532.8 \pm 1{,}798.2$ $\mu m^2/h$ versus to $1{,}619.8 \pm 291.3$ $\mu m^2/h$). Consequently, the highest value of K_p (permeability rate constant) corresponded to piroxicam in formulation in petrolatum + transcutol P 10%. From a comparative point of view, the permeability constants K_p (Table 1) ranged from 0.000976 ± 0.000065 μm/h for propylene glycol to 0.00239 ± 0.000017 μm/h for petrolatum + transcutol P 10%. It has been reported that transcutol P might have the same enhancer effect on the percutaneous absorption of dexamethasone and hydrocortisone [Ritschel et al., 1991] and cyanophenol [Harrisson et al., 1996]. Differences were also found for lag time T_{lag} (Table 1). The shortest T_{lag} was shown for piroxicam in petrolatum + transcutol P 10% ($T_{lag} = 3.02 \pm 0.46$ h), followed by PEG 400 ($T_{lag} = 13.7 \pm 2.8$ h), transcutol P ($T_{lag} = 15.03 \pm 1.23$ h) and propylene glycol ($T_{lag} = 19.3 \pm 2.08$ h) representing the longest lag time (Table 1). These differences in lag time are probably due to the lower coefficients of diffusion D_m (around 2,000 $\mu m^2/h$) across the skin formulation of piroxicam in these vehicles (PEG 400, transcutol P and propylene glycol).

In spite of the small values of D_m and K_p, propylene glycol allowed piroxicam to permeate in large amounts through the skin, and to target the epidermis compartment, with the highest amount of piroxicam (134 ± 47 $\mu g/mg/cm^2$), which was twofold greater than that obtained from petrolatum + transcutol P 10% (Fig. 2). According to the calculated values of permeation parameters (Table 1), the high partition coefficient K_m value as well as the moderate solubility of piroxicam in propylene glycol may be responsible for the high diffusion of piroxicam through the skin. Indeed, the lower the affinity of a vehicle for the drug it contains, the more percutaneous absorption of the drug should occur. In addition, the vehicle propylene glycol has been reported in the literature to increase the percutaneous absorption of piroxicam [Okuyama et al., 1999; Santoyo et al., 1996]. Nevertheless, in spite of the notable penetration of piroxicam, the diffusion of piroxicam from formulation containing propylene glycol to skin was slower than expected. This led to an increase of lag time ($T_{lag} = 19.3 \pm 2.08$ h) compared to most of the other vehicles studied (Table 1). This can be associated with the mechanism of action of propylene glycol in fluidising the skin membrane to allow the permeation of drugs.

Although the higher solubility of piroxicam was found in PEG 400 (23.1 mg/mL) and in transcutol P (13.9 mg/mL), the diffusion was low, with amounts of piroxicam in epidermis, dermis, and receptor liquid never exceeding 27 $\mu g/mg/cm^2$ (Fig. 2). The high solubility of piroxicam in both vehicles could explain this low and slow diffusion of piroxicam, as Okamoto et al. described it [Okamoto et al., 1990]. Otherwise, as shown in Table 1, the low partition coefficient K_m of piroxicam in PEG 400 (0.000216 ± 0.000047) and the stronger binding force between piroxicam and PEG 400 in the formulation would reduce the drug release from the formulation to the skin and therefore would affect the diffusion of piroxicam through the skin. It has been reported that PEG 400 might have the same effect on the percutaneous absorption of piroxicam from dermatological bases [Csoka et al., 1999; Babar et al., 1990]. The situation for transcutol P is similar to that found with PEG 400. The K_m value (0.00020 ± 0.000023) indicated that piroxicam was easily released from the formulation containing transcutol P. However, the diffusion of piroxicam was low, as shown in Table 1 by the lowest value of D_m (1962 ± 153 $\mu m^2/h$) and the low value of K_p (0.000993 ± 0.00008 μm/h). These results are in accordance with the study of Ritschel et al. They reported that hydrocortisone associated with transcutol P did not move readily out of the skin and tended to form skin reservoirs or depots in the stratum corneum, limiting the cutaneous diffusion of hydrocortisone [Ritschel et al., 1991].

As shown by the diffusion profiles achieved using Franz diffusion cells, piroxicam did not permeate in the receptor fluid from the formulation containing petrolatum, and tended to stay in the epidermis, where the amount of piroxicam was 74 ± 37 $\mu g/mg/cm^2$. Nevertheless, this concentration is probably insufficient in epidermis to allow the type IV hypersensitivity reaction as demonstrated by the low sensitivity of in vivo patch

tests [Barbaud et al., 1998]. On the other hand, propylene glycol and petrolatum + transcutol P 10% should be able to induce a sufficient level of exposure for the cells of the immune system at junction epidermis/dermis, suggested by the amounts of piroxicam obtained at 48 h in the different compartments and by the high values of exposure index.

ACKNOWLEDGMENTS

The authors thank the Department of Plastic Surgery of Henri Mondor Hospital for their contribution to this work, especially Mrs. Laurent.

REFERENCES

Abe T. 1982. Piroxicam as a new adjunct for the treatment of rheumatoid arthritis. Am J Med 72:39–44.

Alanko K, Stubb S, Reitamo D. 1987. Topical provocation of fixed drug eruption. Br J Dermatol 116:561–567.

Babar A, Solanki UD, Cutie AJ, Plakogiannis F. 1990. Piroxicam release from dermatological bases: in-vitro studies using cellulose membrane and hairless mouse skin. Drug Dev Ind Pharm 16: 523–540.

Bach M, Lippold BC. 1998. Percutaneous penetration enhancement and its quantification. Eur J Pharm Biopharm 46:1–13.

Barbaud A, Reichert-Penetrat S, Trechot P, Jacquin-Petit MA, Ehlinger A, Noirez V, Faure GC,Schmutz JL, Bebe MC. 1998. The use of skin testing in the investigation of cutaneous adverse drug reactions. Br J Dermatol 139:49–58.

Carrabba M, Paresce E, Angelini M, Galanti, Marini MG, Cigarini P. 1995. A comparison of the local tolerability, safety and efficacy of meloxicam and piroxicam suppositories in patients with osteoarthritis: a single-blind, randomized, multicentre study. Curr Med Res Opin 13:343–355.

Csoka G, Racz I, Marton S, Balogh E, Farkas E. 1999. Formulation of novel soft-patch type gel systems containing non-steroidal anti-inflammatory drugs. Pharm Ind 61:88–91.

Gerber D. 1987. Adverse reactions of piroxicam. Drug Intell Clin Pharm 21:707–710.

Guillaume JC, Roujeau JC, Chevais M, Albengres E, Revuz J, Touraine R. 1985. Syndrome de Lyell et ectodermose pluriorificielle au cours de traitements par les oxicams: 11 observations. Ann Dermatol Venereol 112:807–812.

Harrison JE, Watkinson AC, Green DM, Hadgraft J, Brain K. 1996. The relative effect of Azone® and Transcutol® on permeant diffusivity and solubility in human stratum corneum. Pharm Res 13:542–546.

Katoh N, Kagawa K, Yasuno H. 1995. Piroxicam induced Stevens-Johnson syndrome. J Dermatol 22:677–680.

Kim YH, Ghanem AH, Higuchi WI. 1992. Model studies of epidermal permeability. Semin Dermatol 11:145–156.

Milpied-Homsi B. 1995. Dermites de contact aux anti-inflammatoires non stéroïdiens topiques. GERDA 1:89–101.

Okamoto H, Muta K, Hashida M, Sezaki. 1990. Percutaneous penetration of acyclovir through excised hairless mouse and rat skin: effect of vehicle and percutaneous penetration enhancer. Pharm Res 7:64–68.

Okuyama H, Ikeda Y, Kasai S, Imamori K, Takayama K, Nagai T. 1999. Influence of non-ionic surfactants, pH and propylene glycol on percutaneous absorption of piroxicam from cataplasm. Int J Pharm 186:141–148.

Phelip X. 1984. La tolérance du Feldène® (piroxicam)—bilan de trois études coopératives concernant 48 353 observations. Gaz Med 91:93–96.

Pitts NE. 1982. Efficacy and safety of piroxicam. Am J Med 72: 77–87.

Ritschel WA, Panchagnula R, Stemmer K, Ashraf M. 1991. Development of an intracutaneous depot for drugs. Skin Pharmacol 4:235–245.

Santoyo S, Arellano A, Ygartua P, Martin C. 1996. In vitro percutaneous absorption of piroxicam through synthetic membranes and abdominal rat skin. Pharm Acta Helv 71: 141–146.

Skelly JP, Shah VP, Maibach HI, Guy RH, Wester RC, Flynn G, Yacobi A. 1987. Report of the Workshop on Principles and Practices of In Vitro Percutaneous Penetration Studies: relevance to bioavailability and bioequivalence. Pharm Res 4:265–267.

Stern RS, Bigby. 1984. An expanded profile of cutaneous reactions to nonsteroidal anti-inflammatory drugs. JAMA 252:1433–1437.

Twomey TM, Bartolucci SR, Hobbs DC. 1980. Analysis of piroxicam in plasma by high-performance liquid chromatography. J Chromatogr 183:104–108.

Vasconcelos C, Magina S, Quirino P, Barros MA, Mesquita-Guimaraes J. 1997. Cutaneous drug reactions to piroxicam. Contact Dermatitis 39:145.