Drug Development and Industrial Pharmacy, 2011; 37(5): 613–617

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2010.534098

informa
healthcare

ORIGINAL ARTICLE

Quantification of dermal and transdermal delivery of meloxicam gels in rabbits

Mayurkumar Patel[1,2], Abhay Joshi[1], Hana Hassanzadeth[1,3], Ravi Juluru[1], and Grazia Stagni[1]

[1]Division of Pharmaceutical Sciences, Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY, USA, [2]Current affiliation: International Trading Pharmaceutical Laboratories Inc, Paterson, NJ, USA, and [3]Division of Pharmacokinetics and Drug Therapy, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

Abstract

Background: This study was designed to quantify the effects of penetration enhancers on systemic bioavailability of 0.3% meloxicam (MLX) hydroxypropylcellulose gels. Cutaneous microdialysis was also performed to assess dermis availability and to better understand the penetration process. The gels tested were a 1% oleic acid gel, a 5% menthol gel, and a control gel without penetration enhancers.

Methods: To assess systemic bioavailability, three female rabbits received according to a crossover design 0.135 g/cm^2 of gel applied to a 7.5 × 7.5 cm area of their shaved back and a short (5 min) infusion of 1 mg. In each experiment, blood samples were collected serially for 36 h and analyzed by a validated HPLC method. For skin bioavailability studies, 0.135 g/cm^2 of the same gels were applied to a 1 × 2 cm area on top of a microdialysis probe previously inserted in the dermis. Dialysate samples were collected for 6 h every 30 min.

Results: Systemically, the 5% menthol gel delivered 3.93 ± 0.85 mg of MLX versus the 1.41 ± 0.24 mg of the oleic acid gel. Only traces of MLX were detectable from the control gel. In dermis, substantial concentrations of MLX were detected only after the application of the menthol gel, whereas skin concentration from the control gel and the 1% oleic acid gel were always below the lowest limit of quantification (LLOQ).

Conclusions: The 5% menthol gel can possibly deliver therapeutically relevant amount of MLX *in vivo*. Dermis concentrations can be predictive of systemic plasma levels.

Keywords: Meloxicam, menthol, microdialysis, transdermal delivery, bioavailability

Introduction

Meloxicam (MLX) is a nonsteroidal, anti-inflammatory drug (NSAID) mostly prescribed for symptomatic treatment of rheumatoid arthritis and osteoarthritis. MLX blocks the cyclooxygenase enzymes COX-1 and COX-2 that are involved in the synthesis of prostaglandins, resulting in reduction of inflammation and its accompanying symptoms. Although generally well tolerated, all the NSAIDs may cause major gastrointestinal side effects that sometimes are life-threatening (Wolfe et al., 1999). Injury to gastrointestinal mucosa may be directly produced by the acidity of the MLX molecules or by the inhibition of prostaglandin synthesis. Indeed, several NSAIDs have been formulated for topical application (Heyneman et al., 2000) and are successfully used in clinical practice with decreased adverse effects and improved patient compliance. In addition, it has been demonstrated that NSAIDs promote local analgesia when administered topically through the skin (O'Hanlon et al., 1996).

A 0.3% MLX hydroxypropylcellulose (Klucel®) gel was developed in our laboratory as a potential transdermal delivery formulation for MLX (Jantharaprapap and Stagni, 2007). The effect of three levels of the penetration enhancers, dimethyl sulfoxide, Tween 20, oleic acid, and menthol, were tested in *in vitro* Franz cells using human cadaver skin as membrane. Out of all enhancers studied,

Address for Correspondence: Grazia Stagni, Division of Pharmaceutical Sciences, Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, 75 DeKalb Ave., Brooklyn, NY, 11201, USA. E-mail: gstagni@liu.edu

(Received 06 July 2010; revised 03 October 2010; accepted 17 October 2010)

menthol at the concentration of 5% w/w showed maximum permeation effect with an enhancement ratio of 27.5 compared with the control gel followed by menthol at the concentration of 2.5% w/w with an enhancement ratio of 10.3 and by the 1% oleic acid gel with an enhancement ratio of 6.1.

The purpose of this study was to measure *in vivo* the actual systemic bioavailability of the developed gels. Indeed, the ability of menthol to actually improve skin penetration *in vivo* is controversial (Brain et al., 2006). Specifically, the 5% menthol and the 1% oleic acid were tested and compared with the control gel *in vivo*, in a rabbit model. In addition, the local bioavailability in dermis was measured by cutaneous microdialysis to evaluate the role of dermis in the absorption process of the gels.

Materials and methods

Chemicals

MLX was a gift from Boehringer, Ingelheim, Biberach, Germany. Piroxicam (internal standard), disodium ethylenediaminetetraacetic acid (Na_2EDTA), sodium hydroxide, and oleic acid were from Sigma-Aldrich, St. Louis, MO. Lactated Ringer's injection USP was from Hospira, Inc., Lake Forest, IL. L-Menthol (3-P-menthol) crystal and propylene glycol were purchased from Spectrum Chemical Mfg. Corp., New Brunswick, NJ. Klucel® (4500–6000 Centipoises) was from Hercules Inc., Wilmington, DE. All chemicals were of HPLC or analytical grade.

Quantification of MLX

Apparatus

The HPLC apparatus consisted of a Hitachi L-2130 HTA pump (Hitachi, High Technology, Inc. Schaumburg, IL), a Waters 717 plus Autosampler (Waters Associates, Milford, MA), an Hitachi L-4250 UV–Vis Detector and a Perkin Elmer Nelson 900 Series Interface. Analyte peaks were recorded and integrated using the TurboChrom software Version 6.1 (Perkin Elmer, Waltham, MA).

Dialysate samples

Separation was achieved on a C18 column (SGE, Scientific Instrument Services, Ringoes, NJ, 150 × 4.6 mm, 3 µm particle size). The mobile phase consisted of methanol:water:phosphoric acid in the ratio of 69.9:30:0.1 v/v%. The final pH of the mobile phase was 2.6 to 2.8. Separation was carried out at a flow rate of 0.5 mL/min at room temperature. Autosampler's temperature was kept at 12°C. Detection wavelength was 370 nm. The injection volume was 20 µL. Calibration curves were linear in the range of 0.100–10 µg/mL ($r^2 > 0.99$). The CV (%) for inter-day assays at 0.100 µg/mL (lowest limit of quantification (LLOQ)) and 10 µg/mL were 2.8 and 0.59, respectively. LOD was 5 ng/mL.

Plasma samples

A new method was developed for plasma extraction of MLX: 100 µL of plasma were added with 50 µL of internal standard (Piroxicam) and 100 µL of 1 M HCL. After mixing for 15 min, 1 mL of chloroform was added to the mixture, vortexed for 2 min, and centrifuged at 10,000 rpm for 10 min at 12°C. The upper layer was discarded and the lower layer was transferred to a 1.5-mL vial and evaporated under a gentle stream of nitrogen at 40°C. The residue was re-dissolved in 100 µL of mobile phase, and 30 µL were injected. To calculate the extraction efficiency, the procedure was repeated three times at for a high, medium, and low concentrations (10, 5, and 1 µg/mL). Recovery of MLX from plasma was compared with recovery from standard solutions. Results showed that recovery was above 85% for all concentrations and independent of concentration in the range tested.

Extracted samples were injected onto a C18 column (Ascentis™ C18 HPLC column, 10 cm × 4.6 mm, 3 µm particle size) and eluted at a flow rate of 1.0 mL/min. The mobile phase consisted of methanol, water, and phosphoric acid (59.9:40:0.1% v/v). The UV detector's wavelength was set at 370 nm. The temperature of the autosampler was kept at 12°C. Calibration curves were linear in the range of 0.050–10 µg/mL ($r^2 > 0.99$). The CV (%) for inter-day assays at 0.050 µg/mL (LLOQ) and 100 µg/mL were 12 and 5.5, respectively. The LOD was 25 ng/mL.

Gel formulations

Gels (0.3% w/w MLX) were prepared according to the procedure described by Jantaraprapap and Stagni (2007). For this project, the gels selected were (i) the control gel (no penetration enhancers), (ii) the 1% oleic acid, and (iii) the 5% menthol.

Microdialysis

Microdialysis equipment consisted of a CMA/102 microdialysis pump. Disposable, linear probes were made in our laboratory in accordance with the procedure described by Stagni et al. (1999). They consisted of two 7-cm arms made of polyamide tubing and a 1-cm long semipermeable hollow membrane made of polyacrylonitrile having a molecular weight cutoff of 50 kDa (AN69 HF, Hospal-Gambro, Inc., Lakewood, CO, USA). Lactated Ringer's solution was used as perfusing solution. Flow rate was 1 µL/min and sampling period was 30 min. The capability of the microdialysis technique to recover MLX was tested *in vitro* according to the procedure described by Stagni and Shukla (2003). Experiments were repeated three times at three concentrations (500, 5000, and 10,000 ng/mL). *In vitro* probe gain and loss (de Lange et al., 2000) were 52.1 ± 4.3 ($n = 9$) and 46.9 ± 12.4 ($n = 9$), respectively. Gain and loss were independent of concentration over the range tested.

In vivo experiments

All experiments were approved by the Institution Animal Care and Use Committee (IACUC) at Long Island University, Brooklyn, New York. The experiments were performed in nine females, pathogen-free New Zealand

albino rabbits weighing 3.5–5 kg. The rabbits were held under the standard laboratory condition and were fed with normal chow and given regular tap water for drinking. The age of rabbits was 12–15 months at the time of the experiments. The dorsal skin of rabbits was shaved with the help of an electrical animal hair clipper. A large area of the rabbit's dorsum was shaved 1 day before the experiments. The skin was checked for cuts and wounds by magnifying glass before the application of the gels. Rabbits were tranquilized with an intramuscular injection of 5 mg/kg acepromazine maleate injection (Boehriger Ingelheim Vetmedica, Inc., St. Joseph, MO).

Systemic availability experiments

The systemic bioavailability of the gels was studied in three rabbits. Each rabbit received one application of each gel and one i.v. infusion according to a crossover design.

After the tranquilization took place, an indwelling catheter (Exel Safelet Cath 24 G × ¾) was inserted in the auricular artery for blood collection. The MLX gel (7.5 g) was applied to a 7.5 × 7.5 cm shaved area bordered with EZ-tape (3 M, St. Paul, MN) having a thickness of 1 mm. The gel was carefully distributed over the surface to an even layer. Gels were left in place for 24 h. Blood samples were collected at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 15, 18, 21, 24, 27, 30, and 33 h. Approximately 0.5 mL blood were collected into K_2-EDTA tubes and stored in ice until plasma was separated by centrifugation at 3400 rpm for 15 min (5°C). Plasma samples were stored at –20°C.

The 1 mg MLX i.v. infusion (Metacam injection, 5 mg/mL; Boehriger Ingelheim Vetmedica Inc.) was administered for 5 min with a Harvard Pump (model-PHD-2000) via a butterfly needle inserted in the peripheral vein of the other ear. Blood samples were collected at 0, 5, 15, 30, 60, min and 2, 4, 6, 8, 12, 16, 20, 22, and 24 h.

Skin availability (microdialysis)

The local bioavailability study was performed in six rabbits. After tranquilization took place, two microdialysis probes were inserted according to the procedure described by Stagni and Shukla (2003). Care was taken that needle was inserted in the dermis and apart from blood vessels. After placing the probes, the inlet and outlet of the probe into the skin were sealed with cynoacryl glue to prevent drug penetration through this path. The skin was allowed to recover from the insertion trauma for about 45 min, then on top of one of the probes a uniform layer of MLX gel (0.135 g/cm^2) was placed in a 1 × 2 cm well carved inside a 3 × 4 cm adhesive tape (3M, St. Paul, MN). This probe was then perfused with lactated Ringer's solution at 1 μL/min, and samples were collected every 30 min for 6.5 h. The other probe site was used for retrodialysis to estimate the probe recovery (Stagni and Shukla, 2003). This probe was perfused with a 1 μg/mL MLX solution at 1 μL/min flow rate for 6 h. Samples were collected every 30 min. In some preliminary experiments, this probe was perfused with plain lactated Ringer's solution to verify that the MLX absorbed from the gels would not distribute to skin and affect the skin concentrations.

Data analysis

Calibration curves were analyzed by linear regression. Means, standard deviations, CV% and E%, and noncompartmental pharmacokinetic analysis were performed using Microsoft Office Excel 2007 (Microsoft Corp., USA). The amount of MLX absorbed from the gel formulations was calculated for each rabbit as:

$$F \times \text{Dose} = \text{CL} \times \text{AUC} \quad (1)$$

where clearance (CL) was calculated from the short i.v. infusion and AUC was the area under the plasma concentration resulting from the application of the gels (Stagni and Shukla, 2003).

Results and discussion

This study was designed to quantify the delivery of MLX from three hydroxypropylcellulose gels in dermis and plasma. Menthol (5%) and oleic acid (1%) were selected as penetration enhancers based on a previous *in vitro* study (Jantharaprapap and Stagni, 2007) and because they have different enhancer mechanism as menthol, an alcohol containing terpene (Sapra et al., 2008), acts by possibly destroying the intercellular lipid network of the stratum corneum and by formation of eutectic mixtures, whereas oleic acid may form pools of fluid within the stratum corneum, (Aungst, 1995) and fluidifies the lipid matrix (Francoeur et al., 1990, Naik et al., 1995). Although *in vitro* data are frequently reliable predictor of *in vivo* absorption, there are controversial findings for menthol. Indeed, menthol induces physiological reactions in the living skin such as increased skin temperature and vasodilatation (Brain et al., 2006) that can affect the rate and extent of systemic absorption. Brain et al. (2006) did not observe an enhanced bioavailability in humans when ibuprofen gels were added with 3% menthol. Therefore, in order to better characterize the penetration of MLX from our gels, we measured the concentration of MLX also in dermis by cutaneous microdialysis. Microdialysis is a sampling technique that uses tiny hollow probes with a tubular dialysis membrane that allows compounds of size smaller than the pores to pass throughout it by passive diffusion (Benfeldt, 1999). Microdialysis can sample compounds in the interstitial fluid with minimal tissue damage and under physiological conditions. Measuring the time course of drug concentration in skin allows for a better understanding of the penetration process and of the relationship between skin and systemic bioavailability.

As the amount of drug delivered is usually proportional to the application area, the systemic and skin availabilities were measured in separate experiments. A large application area (7.5 × 7.5 cm) was used for systemic bioavailability studies to simulate a real-life situation and assure detectable plasma levels; conversely, a

small application area (1 × 2 cm) was used for skin availability to avoid the problem of MLX redistribution to the skin that may occur with a larger area. This assumption was verified in a few experiments by inserting a control microdialysis probe in the skin next to the small delivery area. MLX was never detected in the dialysates collected from these probes.

Figure 1 shows the average total plasma concentrations from the three gels tested. MLX appeared in plasma immediately after application of the 5% menthol gel, reached a maximum peak concentration at about 4 h, and maintained a plateau for an additional 2–3 h. Then the concentration decreased according to a bi-exponential decay. Conversely, the MLX plasma concentration following application of the 1% oleic acid gel was detectable only after 1 h, increased slowly up to 9 h, then maintained an almost steady-state concentration for an additional

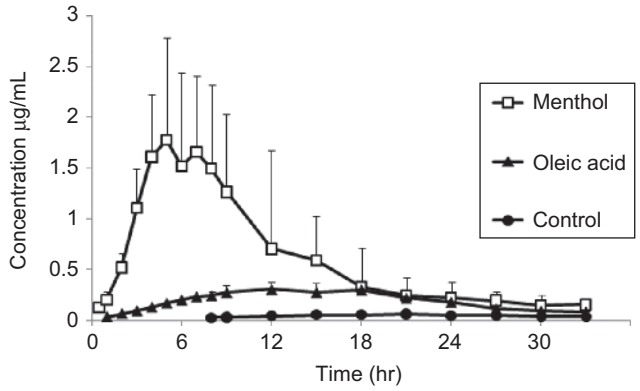

Figure 1. Mean plasma concentration of meloxicam (MLX) from the application of 0.3% MLX gels; (□) 5% menthol, (▲) 1% oleic acid, and (●) control. Error bars represent standard deviation.

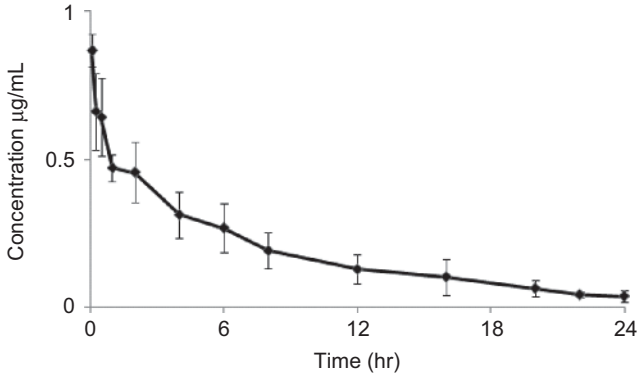

Figure 2. Mean plasma concentration–time profile of meloxicam (MLX) in healthy rabbit following i.v. infusion of MLX ($n = 3$).

10–12 h. Following application of the control gel, MLX was not detectable in plasma for the first 8 h, then its concentrations was detectable but not quantifiable as it never raised above the LLOQ. Data are reported in figure for qualitative significance only.

The short i.v. infusion (Figure 2) was administered in order to calculate the amount of MLX absorbed from the gels. As MLX pharmacokinetics is linear over a wide range of concentrations (Narjes et al., 1996), systemic clearance is independent of the route of administration. Therefore, the amount of MLX delivered by the gels was calculated using Equation 1 and the clearance estimated for each rabbit (Table 1). This is a common assumption that is the starting point for the calculation of fraction of dose absorbed (Gibaldi and Perrier, 1982) in standard bioavailability/bioequivalence studies. Table 1 reports the amount delivered ($F \times$ dose) and the absolute bioavailability fraction (F) for each rabbit. While MLX delivered from the control gel was not quantifiable, the 5% menthol gel delivered 3.93 ± 0.85 mg ($n = 3$) and the 1% oleic acid gel 1.41 ± 0.24 mg ($n = 3$). Therefore, an area of approximately 10 × 10 cm might be able to deliver the necessary 7.5 mg/day of MLX recommended for the treatment of arthritis and osteoarthritis in humans. The relationship between permeability from rabbit skin and human skin is not known but it is reasonable to expect that human stratum corneum is more of a barrier than the shaven rabbit skin and a larger area will probably be necessary. However, an advantage of a gel formulation compared with a pre-filled patch is that it can be easily spread over a larger area.

Only the application of the 5% menthol gel produced quantifiable concentration of MLX in the skin dialysate (Figure 3). MLX was not detectable in skin when 1% oleic acid and the control gel were applied. It should be noted that MLX is 99% bound to plasma proteins (Schmid et al., 1995) mostly albumin, and therefore it is probably highly bound also to the proteins present in the interstitial compartment of the skin. Microdialysis collects only the unbound drug present in the interstitial fluid (Groth, 1998) and high protein or tissue binding is one of the factors that negatively affects probe recovery (Benfeldt and Groth, 1998). Therefore, the concentration of unbound MLX in the extracellular fluid was probably too low to be measurable in dialysate samples from the application of the 1% oleic acid and the control gels. However, it is interesting to note the correlation with the systemic bioavailability experiments (Figure 1)

Table 1. Meloxicam pharmacokinetic parameters in plasma.

	i.v. Infusion		Menthol gel			Oleic acid gel		
Rabbit	Clearance (mL/h)	Half-life (h)	AUC (µg h/L)	F. Dose (mg)	*F*%	AUC (µg h/L)	F. Dose (mg)	*F* (%)
1	262	7.4	12.3	3.22	14	6.19	1.62	7
2	208	6.5	17.8	3.70	16	7.07	1.47	7
3	150	8.4	32.4	4.87	22	7.69	1.16	5
Mean ± SD	207 ± 55	7.5 ± 0.9	20.8 ± 10.4	3.93 ± 0.85	17 ± 4	6.98 ± 0.75	1.41 ± 0.24	6 ± 1

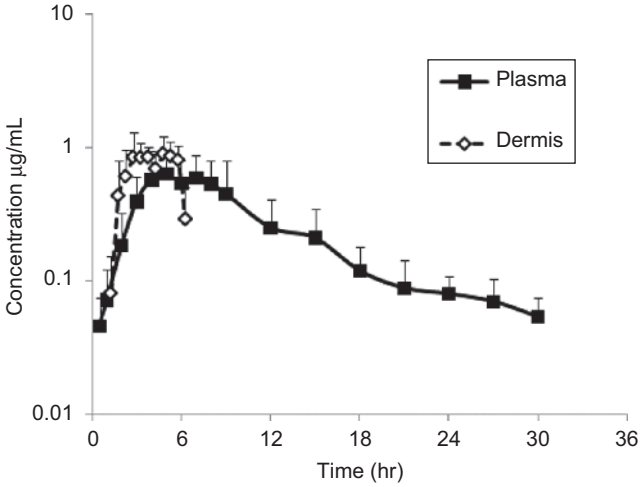

Figure 3. Average unbound concentration of meloxicam in dermis ($n=6$) and plasma ($n=3$) following the application of the 0.3% meloxicam (MLX) gel containing 5% menthol as penetration enhancer. Plasma data were normalized by the size of the delivery area.

where plasma levels were also extremely low during the first 6 h.

In order to compare the skin concentrations detected from the 5% menthol gel application with the plasma concentrations, as in Figure 3, the plasma concentrations were transformed to unbound concentrations (fraction unbound = 0.01) and then divided by 28.125 that is the ratio between the application area of the gel in the systemic availability studies (7.5 × 7.5 cm) and the area for skin availability studies (1 × 2 cm). Figure 3 shows that the skin concentration reaches a plateau at approximately 2–3 hours, remains at plateau for 3–4 h, and drops thereafter. MLX in plasma reaches a plateau with a delay of about 1 h compared with the skin. Therefore, the dermis appears to work as an intermediate (depot) compartment in which MLX accumulates before diffusing to plasma. Figure 3 also shows that both plasma and skin concentration profiles have a large degree of variability when menthol is used as penetration enhancer. This was observed with other NSAID such as ibuprofen (Brain et al., 2006). Indeed, skin availability experiments were performed in six rabbits compared with the three planned at the beginning of the study. It was also observed by visual inspection that when menthol was present, rabbit skin tended to swallow and become hydrated and soft but to a different extent between experiments.

Conclusions

The results of this study confirmed *in vivo* the relative magnitude of delivery as previously found in the *in vitro* study (Jantharaprapap and Stagni, 2007) and that a 5% menthol gel can possibly deliver therapeutically relevant doses of MLX. In addition, the study suggests that dermis dialysate may be predictive of systemic bioavailability.

Acknowledgement

The authors would like to express special thanks to Dr. Joanne van Ryn, Boehringer Ingelheim Pharma (Biberach, Germany) for the donation of MLX powder.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Aungst BJ. (1995). Fatty acids as skin permeation enhancers. Percutaneous Penetration Enhancers. EW Smith, HI Maibach (eds.), CRC Press: Boca Raton, pp. 277–287.

Benfeldt E. (1999). *In vivo* microdialysis for the investigation of drug levels in the dermis and the effect of barrier perturbation on cutaneous drug penetration. Studies in hairless rats and human subjects. Acta Derm Venereol Suppl (Stockh) 206:1–59.

Benfeldt E, Groth L. (1998). Feasibility of measuring lipophilic or protein-bound drugs in the dermis by *in vivo* microdialysis after topical or systemic drug administration. Acta Derm Venereol 78:274–278.

Brain KR, Green DM, Dykes PJ, Marks R, Bola TS. (2006). The role of menthol in skin penetration from topical formulations of ibuprofen 5% *in vivo*. Skin Pharmacol Physiol 19:17–21.

de Lange EC, de Boer AG, Breimer DD. (2000). Methodological issues in microdialysis sampling for pharmacokinetic studies. Adv Drug Deliv Rev 45:125–148.

Francoeur ML, Golden GM, Potts RO. (1990). Oleic acid: its effects on stratum corneum in relation to (trans)dermal drug delivery. Pharm Res 7:621–627.

Gibaldi M, Perrier D. (1982). Pharmacokinetics. Drugs and the Pharmaceutical Sciences. Marcel Dekker, Inc: New York.

Groth L. (1998). Cutaneous microdialysis. A new technique for the assessment of skin penetration. Curr Probl Dermatol 26:90–98.

Heyneman CA, Lawless-Liday C, Wall GC. (2000). Oral versus topical NSAIDs in rheumatic diseases: a comparison. Drugs 60:555–574.

Jantharaprapap R, Stagni G. (2007). Effects of penetration enhancers on *in vitro* permeability of meloxicam gels. Int J Pharm 343:26–33.

Naik A, Pechtold LA, Potts RO, Guy RH. (1995). Mechanism of oleic acid-induced skin penetration enhancement *in vivo* in humans. J Control Release 37:299–306.

Narjes H, Türck D, Busch U, Heinzel G, Nehmiz G. (1996). Pharmacokinetics and tolerability of meloxicam after i.m. administration. Br J Clin Pharmacol 41:135–139.

O'Hanlon JJ, McCleane G, Muldoon T. (1996). Preoperative application of piroxicam gel compared to a local anaesthetic field block for postoperative analgesia. Acta Anaesthesiol Scand 40:715–718.

Sapra B, Jain S, Tiwary AK. (2008). Percutaneous permeation enhancement by terpenes: mechanistic view. AAPS J 10:120–132.

Schmid J, Busch U, Heinzel G, Bozler G, Kaschke S, Kummer M. (1995). Pharmacokinetics and metabolic pattern after intravenous infusion and oral administration to healthy subjects. Drug Metab Dispos 23:1206–1213.

Stagni G, O'Donnell D, Liu YJ, Kellogg DL Jr, Shepherd AM. (1999). Iontophoretic current and intradermal microdialysis recovery in humans. J Pharmacol Toxicol Methods 41:49–54.

Stagni G, Shukla C. (2003). Pharmacokinetics of methotrexate in rabbit skin and plasma after iv-bolus and iontophoretic administrations. J Control Release 93:283–292.

Wolfe MM, Lichtenstein DR, Singh G. (1999). Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 340:1888–1899.