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Bioequivalence determination of topical ketoprofen using a dermatopharmacokinetic approach and excised skin penetration

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Abstract

Ketoprofen is a photolabile drug. The aim of the present study was to compare the bioavailability of ketoprofen in a photo-stabilised formulation with a gel without photoprotection using a new dermatopharmacokinetic tape-stripping model and an established ex vivo penetration method using human skin. Analyses of the stratum corneum showed that during the first 45 min about 12 μg/cm^2 ketoprofen was absorbed into the skin from the formulations. The area under the ketoprofen content–time curve ($AUC_{0-6\,h}$) for the ratio photo-stabilised gel/transparent gel was 73% with a 90% confidence interval (CI) 65–83. The rate of penetration of ketoprofen through isolated skin was approximately 0.2 μg/cm^2 h for both formulations. $AUC_{0-36\,h}$ for the ratio was 84% with 90% CI 64–105. Thus, the two methods did not disagree in terms of relative efficacy of the two gels. However, the difference obtained in vivo was statistically significant, whereas no significant data arise from the ex vivo study. Comparing the amount of ketoprofen in the skin after 45 min with the amount penetrated through the excised skin during 36 h, suggests a change in the thermodynamic activity of ketoprofen during the exposure. A supersaturated formulation may well have been formed initially due to evaporation of ethanol.

Keywords: Ketoprofen; Bioavailability; Dermatopharmacokinetic

1. Introduction

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) ranked to be among the most potent inhibitors of the cyclo-oxygenase pathway of the arachidonic acid cascade, and inhibits lipo-oxygenase as well (Kantor, 1986). The substance penetrates the skin in sufficient amounts to be effective for topical treatment of localized musculoskeletal diseases (Moore et al., 1998; Veys, 1991). A quantitative systematic review of published controlled efficacy studies showed that at least one patient out of three achieved at least 50% reduction in acute pain (soft tissue trauma, strains, and

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sprains) after topical treatment with ketoprofen (Moore et al., 1998).

In controlled clinical studies, local skin reactions and systemic side effects are no more common than with placebo, but several case reports have identified ketoprofen as being responsible for photosensitivity reactions, including both phototoxic and photoallergic adverse effects (Bagheri et al., 2000; Moore et al., 1998). In France 337 cases of photocontact dermatitis attributable to ketoprofen have been identified (Baudot et al., 1998) and in Sweden 42 cases were collected during 2 years (SADRAC, 1998). The frequency in France ranged from 0.0008 to 0.0023% (Baudot et al., 1998) and in Sweden it can be estimated to be almost 0.01%. Sun exposure and young and athletic individuals have been associated to the adverse effects (Baudot et al., 1998).

Ketoprofen is photolabile and ultraviolet (UV) radiation switches ketoprofen into an excited state, where the obtained energy can be dissipated as heat, light or be transferred to the surrounding molecules. If the excitation energy cannot be disposed off, then the molecule may break or rearrange and form reactive oxygen species and toxic decomposition products (Bagheri et al., 2000; Gould et al., 1995). Inclusion of a physical sunscreen filter (titanium dioxide) to a ketoprofen gel has been found effective in reducing the degradation in vitro and in human skin (Lodén et al., 2004). Titanium dioxide scatters and absorbs UV radiation and is commonly used as UV filter in sunscreens. Titanium dioxide is recognized safe in topicals (SCCNFP, 2000). The microparticles do not reach the layer of viable epidermal cells and only small amounts can be located in the follicle orifices after long-term application (Lademann et al., 1999). Thus, the addition of these microparticles is suggested to be of clinical benefit in reducing photosensitivity reactions to ketoprofen.

Whether titanium dioxide will change the bioavailability of ketoprofen in a gel formulation is not known. A pronounced influence on the rate of penetration may reduce the therapeutic advantage with an improved photo-stability of ketoprofen. A changed penetration can be evaluated in efficacy studies and in penetration experiments. However, topical drug products are designed to target the local tissue to which they are applied and, as such, have limited systemic absorption, precluding analyses of blood/urine samples. Moreover, comparative studies on clinical endpoints are relatively insensitive, time-consuming, and costly. Instead dermatopharmacokinetic (DPK) characterization of the penetration of active drugs in human volunteers has been suggested to be able to replace comparative clinical trials as means of documenting bioequivalence (Shah et al., 1998). DPK assessment of drug concentration in the stratum corneum is suggested comparable to blood/urine measurements of systemically administered drugs, where the concentration of a drug in the stratum corneum is expected to relate to its concentrations in viable tissues. Moreover, in vitro methods are encouraged by regulatory agencies with respect to the provision of percutaneous absorption data for drugs, pesticides and cosmetics (Howes et al., 1996). Where differences in clinical endpoints have been shown, permeation rates also vary considerably (Shah et al., 1998).

In the present study the bioavailability of ketoprofen from two gel formulations was compared using DPK characterization in humans and by measuring the penetration ex vivo using excised human skin in flow-through diffusion cells.

2. Material and methods

2.1. Skin

Fifteen healthy volunteers (3 males, 12 females, age 21–27 years, mean 23 ± 1.7 years) with no signs of skin diseases were included in the DPK study, which was approved by the ethics committee at Uppsala University. The study period was June–August. The application areas of the subjects were free from skin diseases, sunburns, tattoos and scars. There were no dropouts among the study population.

Normal looking excised skin from one donor was obtained from breast reduction surgery. Pieces of skin were stored at $-20\,^{\circ}C$ wrapped in aluminum foil.

2.2. Test products

The products were aqueous gels containing 2.5% ketoprofen and about 30% (w/w) ethanol, pH 6–7. One of the gels was photo-stabilised with 4% alumina/silica coated titanium dioxide and hypromellose was used as thickener, whereas the other contained carbomer as thickener (Orudis®, Aventis Pharma). Hypromellose is compatible with titanium dioxide. Menthol was used as

fragrance in the former formulation and lavender oil in the latter.

2.3. In vivo absorption

In the DPK study five areas of 1.8 cm × 2.5 cm were marked on the volar aspect of each forearm. Four areas were synchronously exposed to the gels (2 μl/cm^2) for 45, 90, 180 and 360 min, respectively. The fifth area was a blank. The allocation of study medication to the different positions on the arm was randomised by means of a Latin square with the corresponding area on the other forearm receiving the same dose duration. During the exposure periods the areas were covered with a gauze pad.

After each dose duration 30 tape strips were successively harvested from the treated area with a uniform pressure. The first three strips were considered to represent unabsorbed drug from the skin surface. Tape stripping was used instead of wiping the skin with a wet or dry tissue, since tape stripping was believed to affect the treated area less than wiping of the skin. These three strips, strips 4–7, strips 8–18 and strips 19–30 were collected in four separate vials. In all individuals a non-treated area was stripped and analysed for possible contamination or lateral diffusion of ketoprofen. One single investigator applied and harvested all drug samples.

2.4. Ex vivo penetration

Pieces of skin were mounted in flow-through diffusion cells of stainless steel according to previously described methodology (Loden, 1985; Loden and Faijerson, 1988). The diffusion cells were kept in a water bath at 30 °C. This temperature can be assumed for outer layers of human skin in vivo at room temperature. The area available for diffusion was 0.5 cm^2. Beneath the skin, through the lower section of the cell, the receptor medium (0.01 M phosphate buffer pH 7.4) was pumped and collected in polypropylene vials mounted in a fraction collector. The flow beneath the skin corresponded to about 1–2 receptor volumes per hour. This flow and choice of receptor fluid is considered to ensure proper solubility of ketoprofen and facilitate the partition of ketoprofen from the dermis into the receptor fluid (i.e. sink condition) (Bonina et al., 2001; Ceschel et al., 2002).

The position of the vials was changed every 4 h. To prevent air from collecting under the skin, a bubble trap was placed at the entry of the diffusion chamber. The gel was gently applied to the to the skin with the fingertip. Each gel was tested on seven pieces of skin. Approximately 3 μl was applied to each square centimetre.

2.5. Chemical analysis

The adhesive tapes were extracted with methanol in an ultrasonic bath and centrifuged at 5500 rpm for 10 min. The receptor fluid was evaporated and the precipitate dissolved in methanol and filtered before analysis. The 20 μl of the solution was injected into a reversed-phase high-performance liquid chromatography (HPLC) equipped with a C18 (15 cm × 3 mm, 4 μm particles) column with a UV-detector (Jasco 970 UV/VIS) set at 259 nm, Fig. 1A–C. The mobile

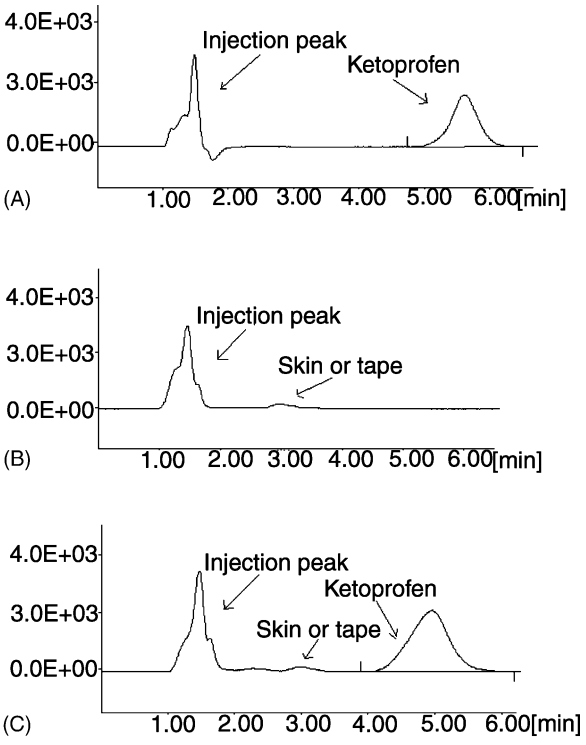

Fig. 1. Representative high-performance liquid chromatography of calibration standard, 2 μg/ml (A), extracted stratum corneum plus adhesive from untreated control site strips 1–3 (B) and 45 min product exposed skin site strips 4–7 (C).

phase was isocratic acetonitrile −0.01 M potassium phosphate pH adjusted to 2.9 with phosphoric acid (40:60 v/v) with a flow rate of 0.5 ml/min (Jasco PU-980 pump).

Unknown amount of ketoprofen in the samples were calculated using calibration standards. The HPLC assay demonstrated accuracy greater than 99% and precision less than 0.3% coefficient of variation. The limit of quantification was 0.12 μg/ml. The standard calibration curve (2–50 μg/ml) demonstrated a mean linear regression parameter of $r^2 = 1.0000$.

2.6. Calculations and statistics

Ketoprofen content was expressed as microgram per centimetre square of adhesive tape surface in the DPK study and as amount penetrated per square centimetre of excised skin and hour in the ex vivo experiment. Areas under the ketoprofen content–time curve (AUC) values were calculated over the 0–*t*-hour time interval (AUC_{0-t}) using the linear trapezoidal rule method.

Median values were calculated and non-parametric statistical tests were applied to the data. The results are presented using box-plots and by calculating the point estimate for the difference along with the confidence interval. The box is defined by the upper and lower quartiles and with the median marked by a subdivision of the box. The whiskers have a maximum length in the terms of the interquartile range and outliers are shown (Minitab Statistical Software, Minitab Inc., PA, USA).

AUC_{0-t} of the two gels was analysed by logarithmic transformation of the data and calculating the Wilcoxon rank sum test together with corresponding two-sided 90% confidence interval (CI) for the ratio of AUC_{0-t}. The gels were considered bioequivalent if the 90% CI for the ratio of the population geometric averages was contained completely within the 80–125% interval. The Wilcoxon signed rank test was used to compare the values at the different dose durations. *P*-values are given and values below 0.05 were considered significant.

3. Results

Analysis of the tape strips showed no signs of contamination or lateral diffusion of ketoprofen into the untreated area, Fig. 1B. Application of the gel formulations to the skin resulted in detectable amounts of ketoprofen in the treated stratum corneum. The content was highest at the surface and then dropped with depth of stratum corneum, Fig. 2A–D. The concentration profiles did not show pronounced differences with time of exposure, although the profile appeared steeper after 45 min of exposure, Fig. 2A–D.

The content in the stratum corneum (tape strips 4–30) was lower after 90, 180 and 360 min of exposure to the photo-stabilised formulation than after treatment with the transparent gel, Fig. 3. The magnitude of the difference in stratum corneum appeared to become more pronounced with time, Fig. 3. This is also reflected as a lower $AUC_{0-360\,min}$ for the photo-stabilised gel during the treatment period ($P = 0.0055$). The ratio of the $AUC_{0-360\,min}$ for the photo-stabilised gel to the ordinary transparent gel was 73% with the 90% CI 65–83.

With increasing lengths of exposure the total amount of ketoprofen found in all tape strips (1–30) showed decreasing levels, Fig. 4. The recovery from the photo-stabilised formulation was higher than from the transparent gel after 45 and 90 min, Fig. 4. Thereafter the difference between the two gels levelled out.

The decrease in the recovery of ketoprofen with time could possibly also be noted as a slight decrease in the outer layer of the stratum corneum (tape strips 4–7) with time, especially for the photo-stabilised gel, Fig. 2A–D.

The penetration of ketoprofen through excised skin showed the same tendency (not significant) to a higher diffusion rate from the transparent gel than from the photo-stabilised version, Fig. 5. However, the ratio of $AUC_{0-36\,h}$ was 84.5% (90% CI 64–105, $P = 0.2013$). The rate of penetration was about 0.2 μg/cm^2 h for both gels and the cumulative amount penetrated was about 7 μg/cm^2 for the photo-stabilised formulation and 9 μg/cm^2 for the ordinary gel. This corresponds to 10% of the applied amount.

4. Discussion

The DPK profile of ketoprofen uptake into human stratum corneum as a function of time demonstrates a rapid attainment of the apparent “steady state”, Fig. 2. The maximum concentration of ketoprofen was reached already within the first 45 min of product ap-

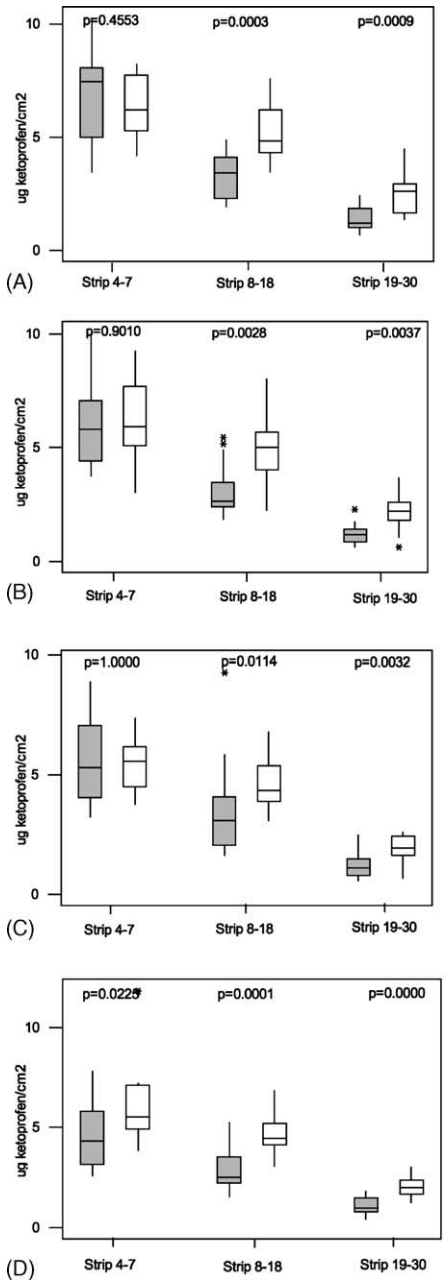

Fig. 2. The amount of ketoprofen at different depths of skin and time of exposure: (A) 45 min, (B) 90 min, (C) 180 min and (D) 360 min; $N = 15$. Grey box: photo-stabilised gel and white box: transparent gel.

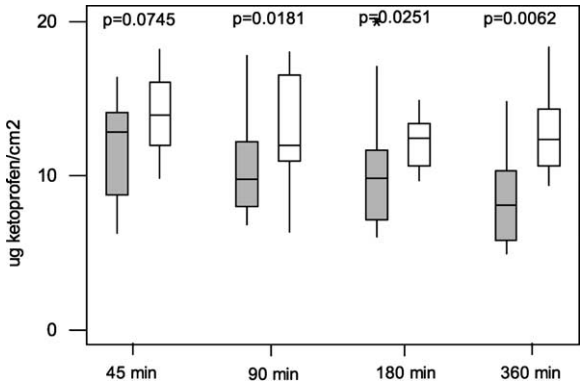

Fig. 3. The amount of ketoprofen in tape strips 4–30 after different exposure times. $AUC_{0-360\,min}$ ratio was 73% with 90% confidence interval 65–83. Grey box corresponds to photo-stabilised gel and white box transparent gel; $N = 15$.

plication, also observed as a similar concentration gradient at 45 min as at the later time points. "Steady state" reflects saturation of the stratum corneum, as well as equilibrium between uptake and elimination of applied ketoprofen from this compartment over time.

A steep concentration gradient throughout the stratum corneum provides the driving force for the diffusion through skin. However, no true steady state can be obtained from a finite dose application due to depletion of the surface concentration with time. This is also reflected in the present study as decreasing amount of ketoprofen on the skin surface with time, which will reduce the driving force for penetration, Fig. 2. Fur-

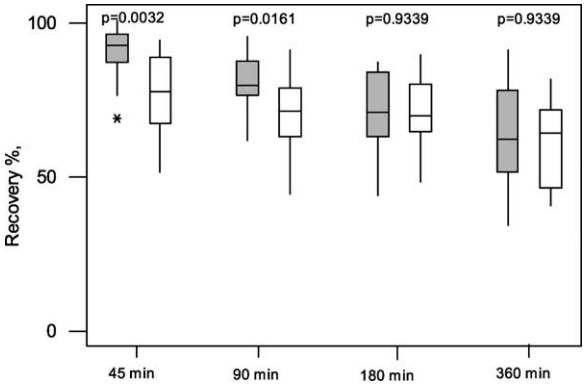

Fig. 4. The recovery of ketoprofen after different exposure times measured as the total amount found in the 30 tape strips. Grey box corresponds to photo-stabilised gel and white box ordinary transparent gel; $N = 15$.

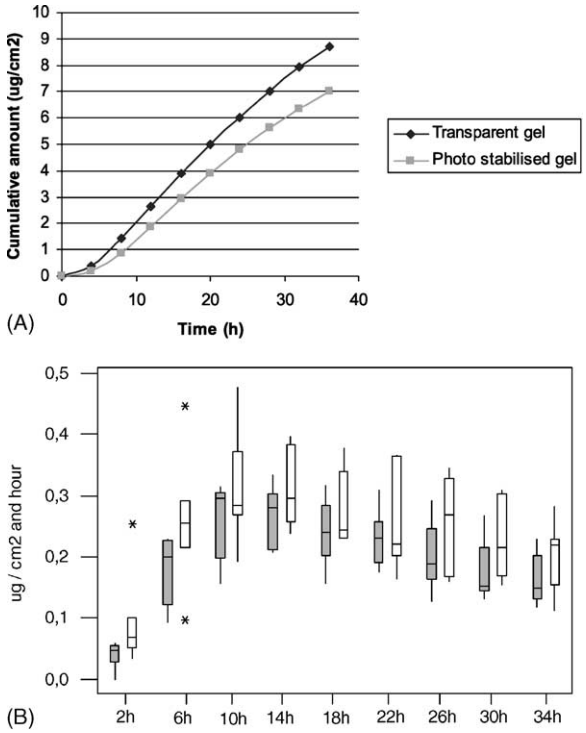

Fig. 5. Diffusion flux (A) and cumulative amount penetrated (B) of ketoprofen through excised human skin. Grey box: photo-stabilised gel and white box: transparent gel; $N = 7$.

thermore, evaporation of volatile agents will change composition with time.

Previous studies suggest that only 50% of applied formulation remains on the surface after 8 h (Rhodes and Diffey, 1997) with observed differences between formulation types (Johnson et al., 1983). This is in agreement with the results in the present study, where about 60% was recovered from the skin after 6 h. Disappearance of ingredients from the surface may be due to absorption into the skin, metabolism, evaporation, sloughing off or by contact with other materials. From urinary excretion data the bioavailability of topically applied ketoprofen has been estimated to approximately 5–9% of the level obtained after an orally administered dose (Shah et al., 1996).

Approximately 10% of the applied dose penetrated the excised skin during 36 h, Fig. 5. The ex vivo penetration curve of ketoprofen showed a longer delay until equilibrium than in vivo, probably due to the presence of dermis. The diffusion curve showed a similar pattern as in other ex vivo studies on human skin and the measured rate of penetration (0.2 $\mu g/cm^2$ h) was within the range of previously reported data. For example, from patches containing 5–10% ketoprofen the diffusion rate through human skin was 0.1–0.3 $\mu g/cm^2$ h (Singh et al., 1996). The flux from 0.5% in ethanol through excised epidermis into an ethanol–water receptor fluid was almost 0.15 $\mu g/cm^2$ h, calculated from a cumulative amount of ketoprofen penetrated (3 $\mu g/cm^2$ during 24 h) (Bonina et al., 2001). Moreover, 2 $\mu g/cm^2$ h penetrated from 1% in a gel (Paolino et al., 2002). Differences still existing between human studies are likely to be due to factors in the experimental set up, different formulations and inter-individual variability in skin permeability (Shah et al., 1996).

In studies on pig skin considerably higher penetration rates have been reported (Ceschel et al., 2002). For 1% gel containing 40% (v/v) ethanol the value was 20 $\mu g/cm^2$ h and for 10 and 20% isopropanol the penetration was 30 and 54 $\mu g/cm^2$ h, respectively (Ceschel et al., 2002). Also rat skin was more permeable and allowed a rate of almost 10 $\mu g/cm^2$ h from a vehicle with 3% ketoprofen (Rhee et al., 2001).

The two collective studies compare in vivo skin stripping data and ex vivo penetration as a function of application duration and composition of two ketoprofen gels. Harvesting and analysing the stratum corneum are viewed as surrogate measures of the clinical efficacy and safety of the formulations (Shah et al., 1998), similarly to systemically administered drugs where effect is related to concentration in the blood. Penetration through excised skin is also a proper method to detect differences between the penetration characteristics of topical formulations (Skelly et al., 1987). The methods did not disagree in terms of relative efficacy of the two ketoprofen gels. However, the difference obtained in vivo was statistically significant, whereas no significant data arise from the ex vivo study. A larger study would be required to achieve statistical power ex vivo. Furthermore, it may be noted that during the first 45 min about 10–15 μg of ketoprofen was absorbed into the stratum corneum in vivo, whereas the diffusion flux through the excised skin was about 0.2 $\mu g/cm^2$. This difference may well reflect an increase in the thermodynamic activity of ketoprofen during evaporation of ethanol, resulting in a supersaturated layer of the drug where the thermodynamic activity is greater than unity. Supersaturated formulations are typically subject to re-

crystallization of the drug substance and concomitant loss of the enhancing effect. Another possible explanation might be a high initial transfollicular absorption in vivo, whereas this route may have been closed ex vivo due to the fully hydrated membrane.

The in vivo study suggests that the anti-logarithm of the AUC for the bioavailability of the photo-stabilised formulation is 73% of that of the conventional gel, with 90% CI 65–83. Thus, they cannot be considered to fulfil the bioequivalence criteria, since this value should fall in the range 80–125% to be considered as bioequivalent (EMEA, 2001). The partition coefficient of ketoprofen between the vehicle and the stratum corneum was expected to be the same in the two gels, since the solubility (or more correct the thermodynamic activity) of ketoprofen in the two vehicles would be the same due to the same concentration of ethanol and the same pH (ca. 30% ethanol with pH 6–7 in both).

The clinical impact of this difference in bioavailability is not known. Although ketoprofen has a broad therapeutic window, it may be anticipated that the analgesic/anti-inflammatory actions of ketoprofen are dose dependent within the limits of the amount delivered topically. Therefore, if all other confounding factors are the same, then it seems reasonable to assume a lower clinical activity of the photo-stabilised formulation than that of the conventional gel. In order to compensate for 27% lower bioavailability the radius of a circular area has to increased by 17%, i.e., for example from 6 to 7 cm.

Differences in percentage skin penetration of another NSAID (5% ibuprofen) in six licensed formulations has recently been reported (Hadgraft et al., 2003). The differences absorbed ranged from 4 to 22% in three gel formulations, up to 25% in a spray, with steady-state fluxes from 0.2 to 4.2 $\mu g/cm^2$ h (Hadgraft et al., 2003) which is far more pronounced differences in magnitude than in the present study (>500% compared to almost 30% in the present study). A clinical review shows that 3.5 patients need to be treated topically with ibuprofen in order for one patient to obtain 50% pain reduction, whereas 2.6 need to be treated to obtain treatment success from topical treatment with ketoprofen (Moore et al., 1998). Hence, the data suggest that a lower bioavailability of ketoprofen may still be of clinical benefit. This is also shown from a clinical and dermatopharmacokinetic study on an FDA licensed tretinoin (Pershing et al., 2003). The new gel showed clinical inferiority to the conventional gel along with 50% lower bioavailability (Pershing et al., 2003). However, in patients with acne the new gel was found efficacious and to be more effective than placebo (Pershing et al., 2003).

In conclusion, the two bioavailability studies were not in conflict with each other, even though the absorption of ketoprofen in the stratum corneum during the first 45 min was far above the rate of penetration through excised skin. This result is compatible with a possible formation of a supersaturated formulation during evaporation of ethanol. Moreover, the results indicate slightly lower bioavailability of ketoprofen from the photo-stabilised formulation, due to a lower AUC in vivo. Thus, the photo-stabilised formulation may be inferior in reducing pain, which may well be accepted when the superior UV stability is put into the clinical context (Lodén et al., 2004).

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