Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) 156–165
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Probing the skin permeation of eicosapentaenoic acid and ketoprofen 2. Comparative depth profiling and metabolism of eicosapentaenoic acid

Christopher P. Thomas, Charles M. Heard *

Welsh School of Pharmacy, Cardiff University, Wales, UK

Received 15 September 2006; accepted in revised form 27 November 2006
Available online 12 December 2006

Abstract

Unexpected enhancement of the topical delivery of eicosapentaenoic acid (EPA) across porcine skin was observed previously when fish oil was co-formulated with ketoprofen. In the current work depth profile analysis was used to probe the epidermal conversion of EPA to its 15-hydroxy metabolite in the presence and absence of ketoprofen. Freshly excised full-thickness porcine skin in Franz diffusion cells was dosed (both infinite and finite) with simple formulations based on fish oil as source of EPA. After 24 h the skin was subjected to tape stripping and depth profiles were constructed. Typical depth profiles were obtained, with an inverse relationship between depth and permeant concentration. 15-HEPE was generated in the skin when Hepes-modified Hanks' balanced salt solution was used, but none was detected when a cetrimide receptor phase was used, highlighting the importance of maintaining skin viability in such exercises. Ketoprofen had a direct influence on the metabolism of EPA and resulting in conversion to its 15-LOX metabolite 15-HEPE. However, this link appears to be only part of the solution of EPA enhancement however, as even in non-viable skin ketoprofen had an enhancing affect.

Keywords: EPA; Metabolism; Skin; Ketoprofen; 15-HEPE; Fish oil

1. Introduction

The principal polyunsaturated fatty acid present in fish oil, eicosapentaenoic acid (EPA), has been found to possess numerous properties beneficial to health [1]. Topical delivery of the oil offers the ability to avoid wide distribution and other pitfalls associated with oral doses [2]. The transcutaneous delivery of EPA from a fish oil vehicle had previously been demonstrated in vitro [3] with potential value in the treatment of arthritis [4]. Furthermore topically applied fish oil was found to have an anti-inflammatory activity against UVB-induced erythema in vivo [5]. The simultaneous delivery of EPA and ketoprofen across skin from a fish oil vehicle has also been demonstrated, where the data also indicated an inter-relationship between the permeation of EPA and ketoprofen. Ketoprofen permeation was shown to be enhanced by EPA and EPA permeation by ketoprofen, which was difficult to explain using regular skin permeation theory. It was thought that epidermal metabolism or inhibition of such metabolism may go some way to rationalizing the data.

Following topical application to the skin polyunsaturated fatty acids are believed to become incorporated within keratinocytes [6]. Incorporation of EPA into cells plays an important role in inflammation as the replacement of arachidonic acid AA results in a decrease in inflammatory mediators. One study showed that after 6 weeks of ingestion of 3.6 g EPA daily, a decrease of arachidonic acid AA composition of neutrophil membranes of 33% was induced [7]. This incorporation would also be expected to occur in keratinocytes. However, the fact that EPA has been demonstrated to traverse skin intact indicates intercellular permeation occurs such that incorporation of the applied dose into keratinocyte membranes does not exclusively take place [8].

* Corresponding author. Welsh School of Pharmacy, Cardiff University, Redwood, King Edward VII Avenue, Cardiff, Wales CF10 3XF, UK. Tel.: +44 29 20875819; fax: +44 29 20874149.
E-mail address: heard@cf.ac.uk (C.M. Heard).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.11.024

When used as penetration enhancers fatty acids have been shown to pool and even form discrete lipid domains within bilayer lipids in the stratum corneum (SC) [9]. From these pools, it is plausible that the fatty acids could cause saturation of enzymatic pathways as enzyme production in excised skin is limited. Therefore, saturation of the endogenous enzymes by a concentrated pool of fatty acids is feasible as opposed to a small constant supply that the in vivo metabolic processes can deal with efficiently. However, with high local bioavailability of EPA it is probable that metabolism takes place to a certain degree possibly to an initial saturation limit before the production of further catabolic enzymes.

The co-permeation of molecules of different species across skin is a recently probed phenomenon and the effect on metabolism exerted by a co-permeant has had little examination. In particular, as EPA and ketoprofen both act upon COX, whether the two actives will act synergistically or competitively with COX in the skin and the extent to which one would enhance or retard the metabolism or function of the other was unknown.

To investigate the metabolism of compounds within the skin it is a vital pre-requisite that the skin be as fresh and as metabolically active as an in vivo experiment would allow. It is important, when designing such investigation, either into the metabolism of a drug or delivery of a co-drug, to ascertain whether the enzyme activity within freshly excised skin, using a non-growth media receptor phase, is equivalent to the activity profile of growth media-sustained skin. The simplest way of providing the growth media to the skin is utilising the growth media as the receptor phase solution in a normal Franz cell permeation experiment.

This paper describes the investigation into the metabolism of EPA by 15-LOX as it permeates through the skin from a fish oil vehicle. It has been proposed that, from oral doses, EPA is metabolised within the skin via epidermal 15-lipoxygenase to its monohydroxylated metabolite: 15-hydroxyeicosapentaenoic acid (15-HEPE) [10]. It has also been reported that these metabolites accumulate in the epidermis of guinea pigs after ingestion of fish oil ethyl esters [11]. 15-HEPE is also a non-potent inflammatory mediator with the ability to actively compete with AA and thus inhibit the production of the 2-series prostaglandins and the 4-series leukotrienes via the COX and LOX pathways. Interestingly, it was found that the 15-HEPE is more potent than EPA at inhibiting cellular growth and AA metabolism in human prostatic cancer cells in culture [12]. It is for this reason that 15-HEPE has been studied, as converted esters or ceramide derivatives have little or no benefit in the eicosanoid cascade.

The effect of the incorporation of ketoprofen into the formulation on the penetration, and conversion of EPA to its 15-LOX metabolite is also examined, to observe if ketoprofen modifies 15-LOX metabolism in any way and to try and explain the increased permeation of EPA seen previously when incorporated within the same formulation. Additionally metabolism is examined using either growth media-sustained skin (GMS) or a non-growth media (NGM) receptor phase solution, 30 mg ml^{-1} aqueous cetrimide. In this context the relative metabolite production would be indicative of the viability of the skin under the different conditions.

2. Materials and methods

2.1. Materials

Fish oil capsules 1000 mg (Boots Super Strength, Batch No. 30446M) were purchased from a local store. Ketoprofen, butylated hydroxyanisole (BHA) and cetrimide BP were obtained from Sigma–Aldrich Company Ltd., Poole, UK. High vacuum grease was from Dow Corning, Barry, UK. Methanol (HPLC grade) was obtained from Fisher Chemicals, Loughborough, UK. All other reagents were of analytical grade or equivalent. D-Squame® Stripping Discs were obtained from CuDerm Corporation, Dallas, Texas. Freshly excised pig ears were obtained from a local abattoir prior to steam cleaning, cleaned under running water and the hair removed using electric clippers. The time interval between slaughter and commencement of experimentation was approximately 1 h. Full-thickness skin was then removed from the dorsal side of the ear, cut into approximately 2×2 cm sections and used within 2 h of excision.

2.2. Depth profile analysis of EPA and ketoprofen

2.2.1. Preparation of formulations

A fixed sub-saturated dose of ketoprofen (2.5% wt/wt) in fish oil was used in each formulation. The appropriate masses of ketoprofen and fish oil were combined, in addition to BHA (0.05% wt/wt), incorporated as a standard reagent, to inhibit PUFA degradation as previously examined [3], and the formulation vortex mixed. The formulations were stored at 2–4 °C until required ($\leqslant$24 h).

2.2.2. Receptor phases

Two different receptor phases were used during these experiments to determine the viability of porcine skin with and without the presence of growth media. In this case Hepes-modified Hanks' buffer solution (HHBSS) was the growth medium of choice as its use has been well documented in prolonging the viability of skin post excision [13]. HHBSS was prepared by the addition of 25 μM of Hepes and 50 μg ml^{-1} of gentamicin sulphate to Hanks buffer solution, prepared as per manufacturer's instructions. Degassed 30 mg ml^{-1} cetrimide solution was the second receptor phase solution utilised.

2.2.3. Depth profiling

Skin permeation experiments were carried out using glass Franz-type diffusion cells with the donor chambers of the cells (diffusional are approximately 1 cm in diameter)

dosed with either 1 ml (approximately 0.8 g) or 30 μl (approximately 30 mg) of each formulation ± ketoprofen. The 30 μl doses were applied to the skin then gently massaged into the skin 10 times using a circular motion of a glass rod. A total of six replicates were carried out for each formulation. The skin was removed from the cell and excess formulation was removed from the top of the skin prior to tape stripping. A D-Squame® adhesive disc (1 cm in diameter) was placed firmly over the diffused area of the skin sample. The strip was then removed using forceps and placed in a clean glass vial. This was repeated using 30 individual strips which were grouped three per vial before methanol (2 ml) was added and the vial left for approximately 24 h under light exclusion on a laboratory shaker. The remaining epidermis was heat separated from the dermis by placing the skin section epidermis side down onto a glass plate maintained at 55 °C for approximately 30 s. The remainder of the epidermis was removed and both the dermal and epidermal tissue placed in separate glass vials with methanol (4 ml) and extracted as above.

Recent works have described tape stripping in conjunction with mass spectroscopic determinations [14], although such were not deemed necessary in the current paper. The receptor phases were not analysed for the presence of permeated EPA or 15-HEPE as the water-based HHBSS employed as receptor phase would not provide adequate sink conditions for the permeation of these lipophilic compounds. The skin permeation of EPA has been examined previously [8].

2.3. HPLC analysis

2.3.1. Ketoprofen

Ketoprofen samples were analysed on a Hewlett Packard 1100 HPLC system with a variable wavelength detector. Separations were carried out on a Phenomenex Kingsorb C18 column (150 × 4.6 mm, 5 μm) fitted with a Phenomenex Securiguard guard column. The mobile phase was acetonitrile/potassium phosphate buffer (pH 1.5) (55:45, v/v) run isocratically for 10 min at a flow rate of 1.0 ml min^{-1}. UV detection was set at 258 nm. The retention time of ketoprofen was 6.8 min. A standard calibration curve was constructed over the range 1–120 μg ml^{-1}, prepared in receptor phase, which provided an r^2 of 0.99. The limit of detection (LOD) for ketoprofen was 0.03 μg ml^{-1}.

2.3.2. EPA and 15-HEPE

EPA and 15-HEPE samples were analysed on the above HPLC system and column. The mobile phase was methanol/water/acetic acid (95:5:0.1, v/v) run isocratically for 20 min at a flow rate of 1.0 ml min^{-1}. Detection was UV at 210 nm. The retention time of EPA was 6.9 min. The retention time of 15-HEPE was 4 min. Standard calibration curves were constructed over the range 1–250 μg ml^{-1}, prepared in extraction solvent, which provided an r^2 of 0.99. The LOD for EPA and 15-HEPE were 1.3 and 0.7 μg ml^{-1} respectively.

2.4. Data analysis

HPLC area data were used to determine the concentration (μg cm^{-2}) of compound and these data were plotted against tape strip number. Statistical analyses were carried out using Instat 3 for Macintosh (GraphPad Software Inc.), where non-parametric Mann–Whitney tests were employed to determine differences between specific pairs of formulations.

3. Results and discussion

3.1. Depth profile analysis of EPA – cetrimide receptor phase

The depth profile analysis for EPA using freshly excised porcine skin and the cetrimide receptor phase (non-growth media) is seen in Fig. 1a. Typical profiles were produced, whereby the amounts of EPA localised, diminished with increasing depth [15] and is consistent with the use of D-Squame® tape strips as it is known that larger amounts of SC are removed by the initial stripping. The profile shows the mass cm^{-2} (±SEM) extracted from the groups of tape strips 1–30 and the remaining heat-separated epidermis and dermis of the skin section.

It must be noted that no 15-HEPE was detected, suggesting either that the skin had ceased to be viable before the experiment or the cetrimide receptor phase solution was adversely affecting metabolic processes within the skin. The results show a slight difference in penetration of the formulations containing ketoprofen and those which do not. A lower concentration of EPA can be expected when ketoprofen is present as there is 2.5% less EPA in the applied dose. The difference in the 30-μl dose and 1-ml dose is as expected, in that the 1-ml dose shows significantly greater permeation ($p = 0.0387$). This is a common feature of finite versus infinite dosing. However, the presence of ketoprofen in the formulation in the 30 μl dose enhances the penetration of EPA through the skin to the lower levels of the epidermis compared to that of the formulation containing no ketoprofen, as illustrated by the increased concentration of EPA in strips 10 to the epidermis. This enhancement, however, is deemed not significant ($p = 0.5899$).

A clearer comparison of doses can be made when examining the percentage of the applied dose that permeated (see Fig. 1b). This takes into account the presence and the true effect of the ketoprofen upon the permeation of EPA. When considering percentage values, it can be seen that proportionately more EPA localised from the 30-μl dose containing ketoprofen than from the 1-ml dose containing ketoprofen equating to an average of 0.6% of the applied dose. This may be related to the massaging protocol employed when applying the 30-μl dose. Massaging

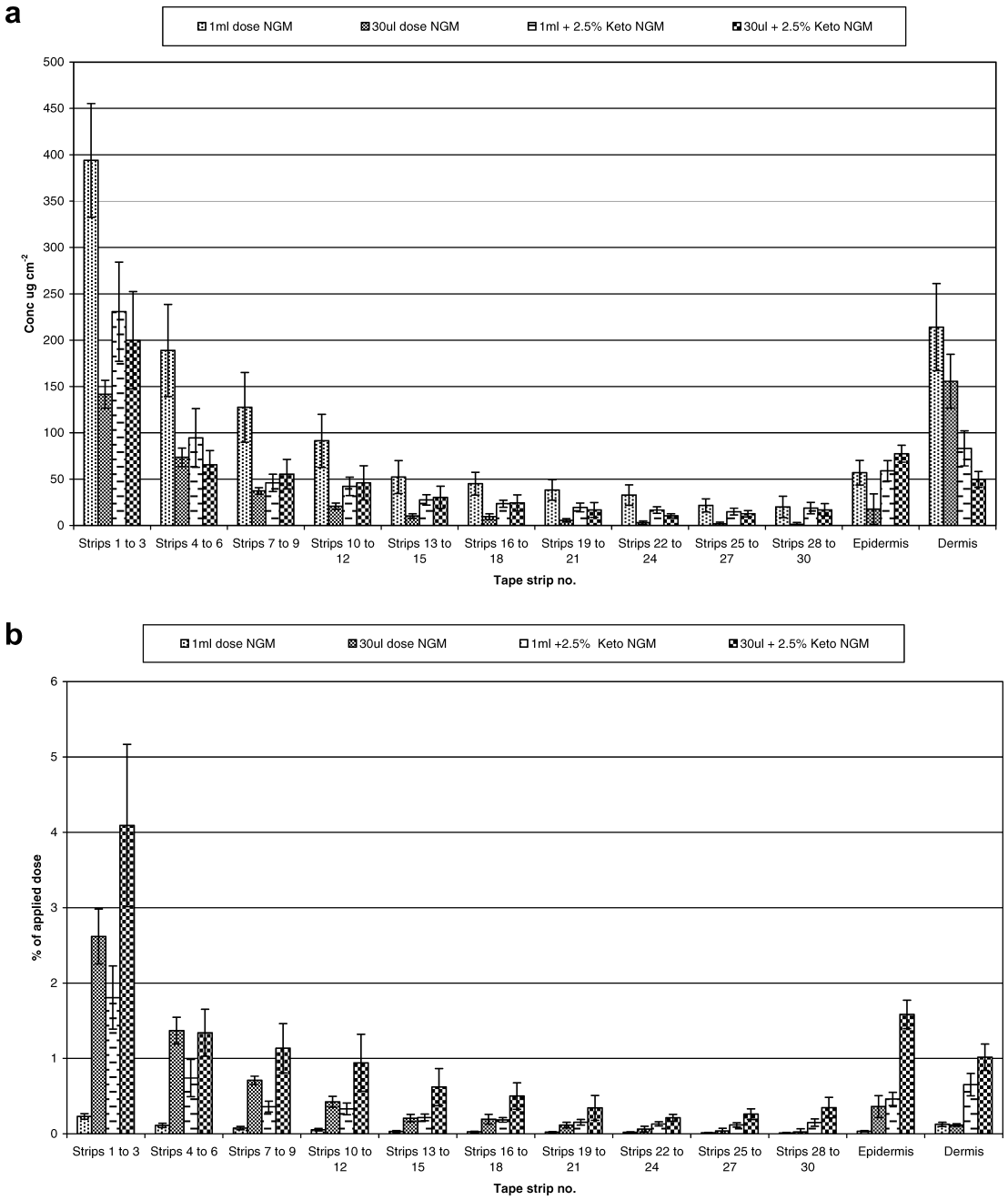

Fig. 1. (a) Concentration of EPA as a function of depth. (b) Percentage of EPA from applied dose of non-growth media samples ($n = 6 \pm$ SEM).

enhances the uptake into skin, possibly assisted by mechanical erosion of the SC.

3.2. Depth profile analysis of EPA – growth media-sustained skin

The results using the growth media-sustained skin show that there was no significant difference ($p > 0.10$) between any of the doses (see Fig. 2a). There was however an overall reduction in the concentration of EPA detected. A direct comparison between the growth media-sustained and non-growth media samples indicated that the amounts of EPA differed by up to 200 μg cm^{-2} in the 1 ml infinite doses compared to the same dose with the NGM samples, producing a difference of 0.05–0.1% of the applied dose thus indicating a significant breakdown of EPA during penetration. This is thought to be due to the greater metabolic activity in the skin as an effect of growth media sustaining the skin, as the other aspects of the experiment were identical.

The formulation that exhibited the greatest penetration was the infinite dose containing ketoprofen. This is to be expected as, if a ketoprofen–EPA complex were to exist, this would affect the metabolism of EPA by the LOX enzymes including, as in this case, the 15-LOX isoform.

There does not appear to be much difference in the concentrations of EPA in the skin between the other

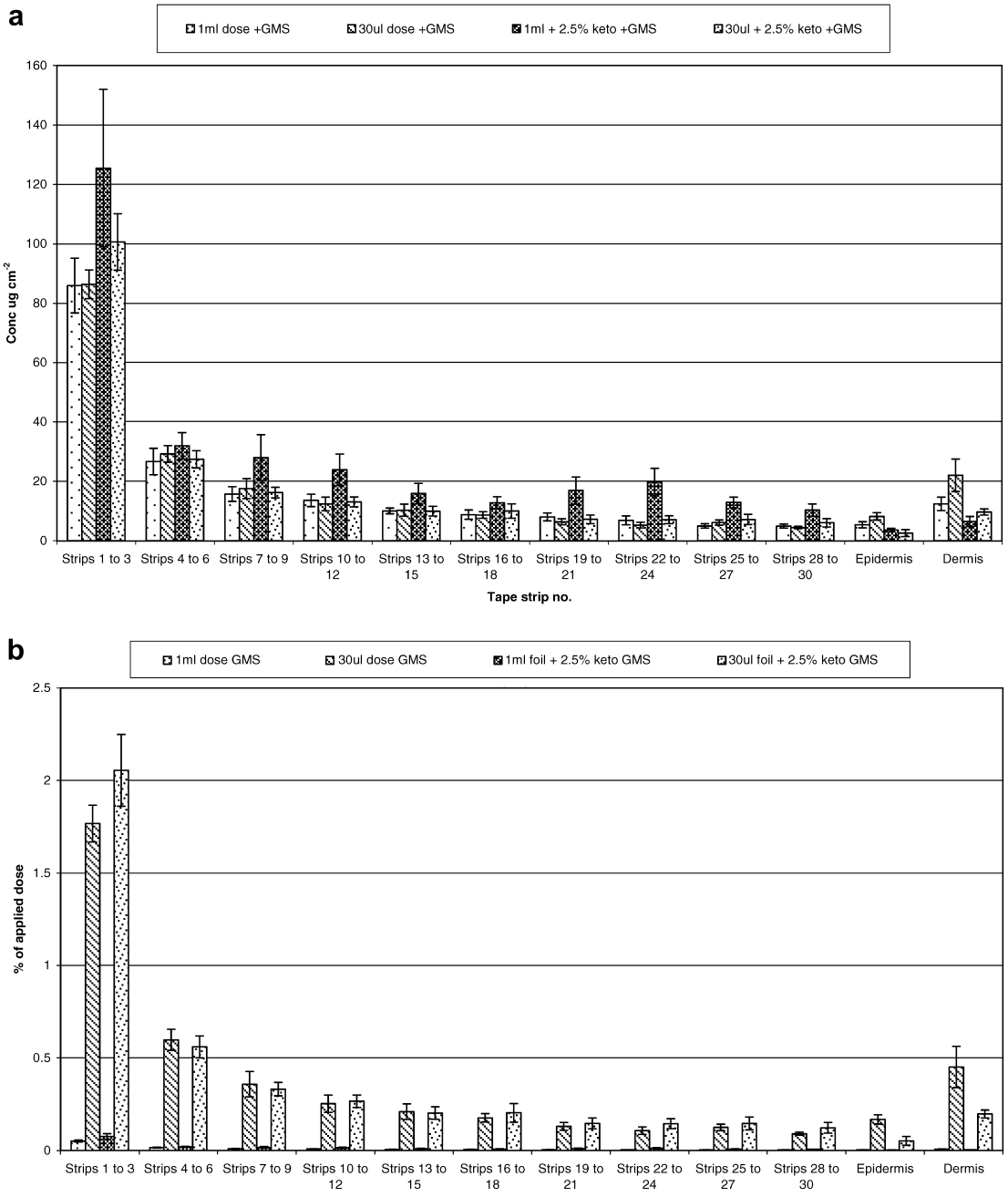

Fig. 2. (a) Concentration of EPA as a function of depth. (b) Percentage of EPA from applied of growth media-sustained samples ($n = 6 \pm$ SEM).

formulations or doses. This could be due to a saturation of the LOX metabolic pathway of EPA, perhaps even at a 30 μl dose. Once again a clearer trend can be seen when the percentage of the applied dose is compared (Fig. 2b).

3.3. Depth profile analysis of ketoprofen

The depth profiles for ketoprofen in all doses are compared in Fig. 3. The majority of the ketoprofen is found in the dermis of all samples after 24 h of penetration. It is clear that the greatest concentration of ketoprofen is present when a 1 ml dose is used with the non-growth media cetrimide receptor phase solution. The growth media-sustained samples show a vastly reduced concentration of ketoprofen in remaining epidermis and dermis, indicating its utilisation within the skin either from an EPA–ketoprofen complex or in its role as an inhibitor of COX enzymes. No ketoprofen was found in the skin of samples dosed with formulations containing no ketoprofen.

Fig. 4 shows the molar ratio of EPA:ketoprofen as a function of depth within the skin. The average molar ratio for 1 ml GMS, 30 μl GMS and 1 ml NGM samples was approximately 10:1. This increases sharply in the 30 μl NGM sample to approximately 34:1. This may be due to

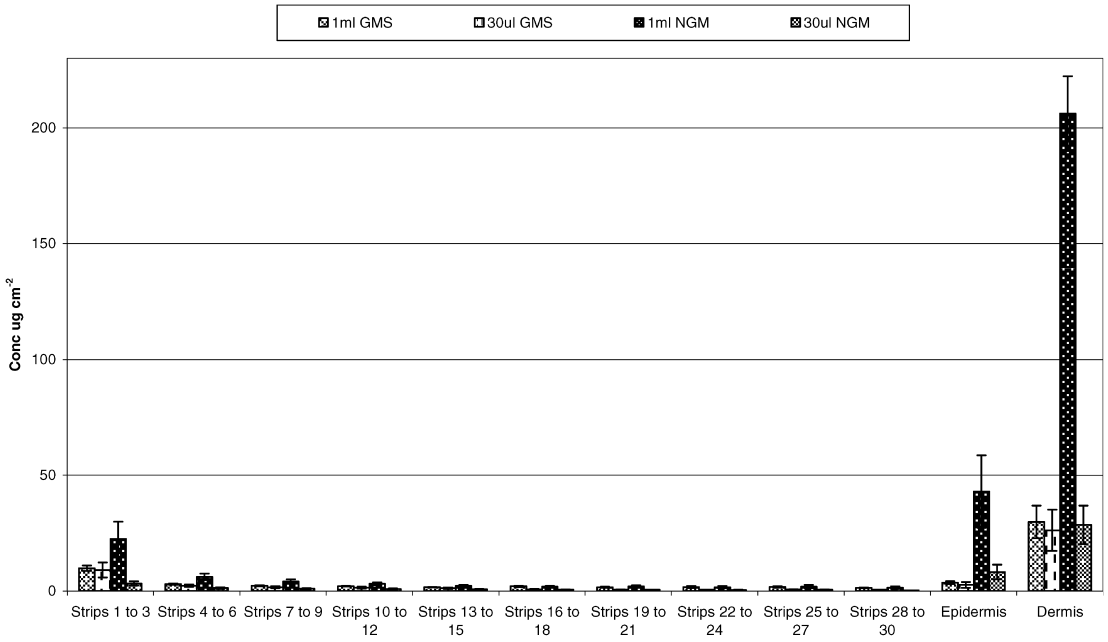

Fig. 3. Concentration of ketoprofen as a function of depth, finite and infinite doses for both growth media-sustained and non-growth media samples.

the readiness and ease of permeation through less metabolically active skin to the dermis, leaving a greater proportion of EPA in the epidermal layers. The molar ratio of EPA:ketoprofen within the formulation was approximately 6:1 which is less than that found within the skin. This may be due to the metabolism of the fish oil triacylglycerols to free fatty acids possibly allowing greater association.

3.4. Analysis of 15-HEPE – growth media-sustained skin

Unlike with the non-growth media samples, Fig. 5a shows that 15-HEPE was being generated in the skin when GMS was used as receptor phase. This suggests:

1. The skin was indeed viable during the experiment,
2. The cetrimide receptor phase may have had an adverse affect upon the skin in the non-growth media samples.

The presence of this metabolite is itself indicative of a high metabolic activity within the GMS skin samples. The distribution of 15-HEPE is consistent with the hypothesis that the presence of ketoprofen is one reason for the increased penetration of EPA, i.e. as a consequence of forming a permeation complex and thus inhibiting the metabolism of EPA by 15-LOX, a hypothesis which has been examined using NMR spectral modulation and molecular graphics [16].

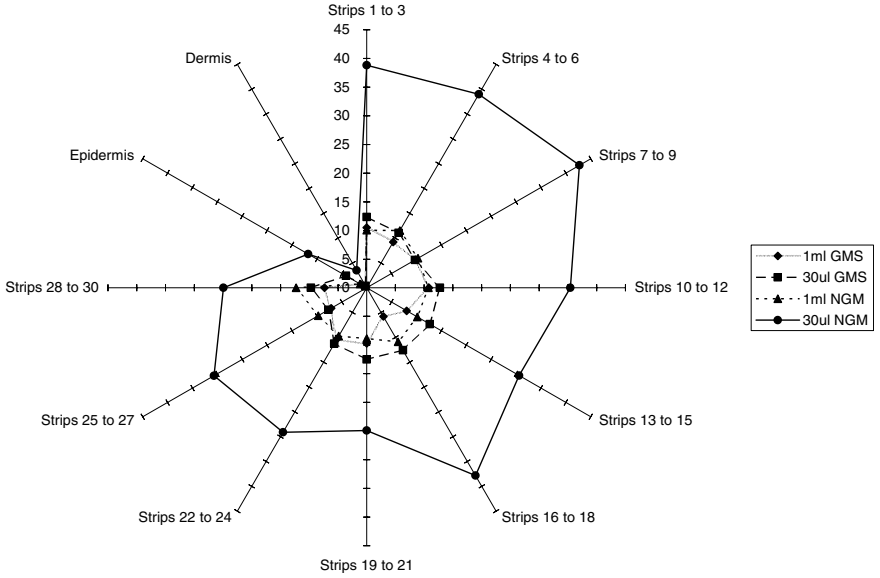

Fig. 4. Molar ratios of EPA:ketoprofen as a function of depth, finite and infinite doses for both growth media-sustained and non-growth media samples.

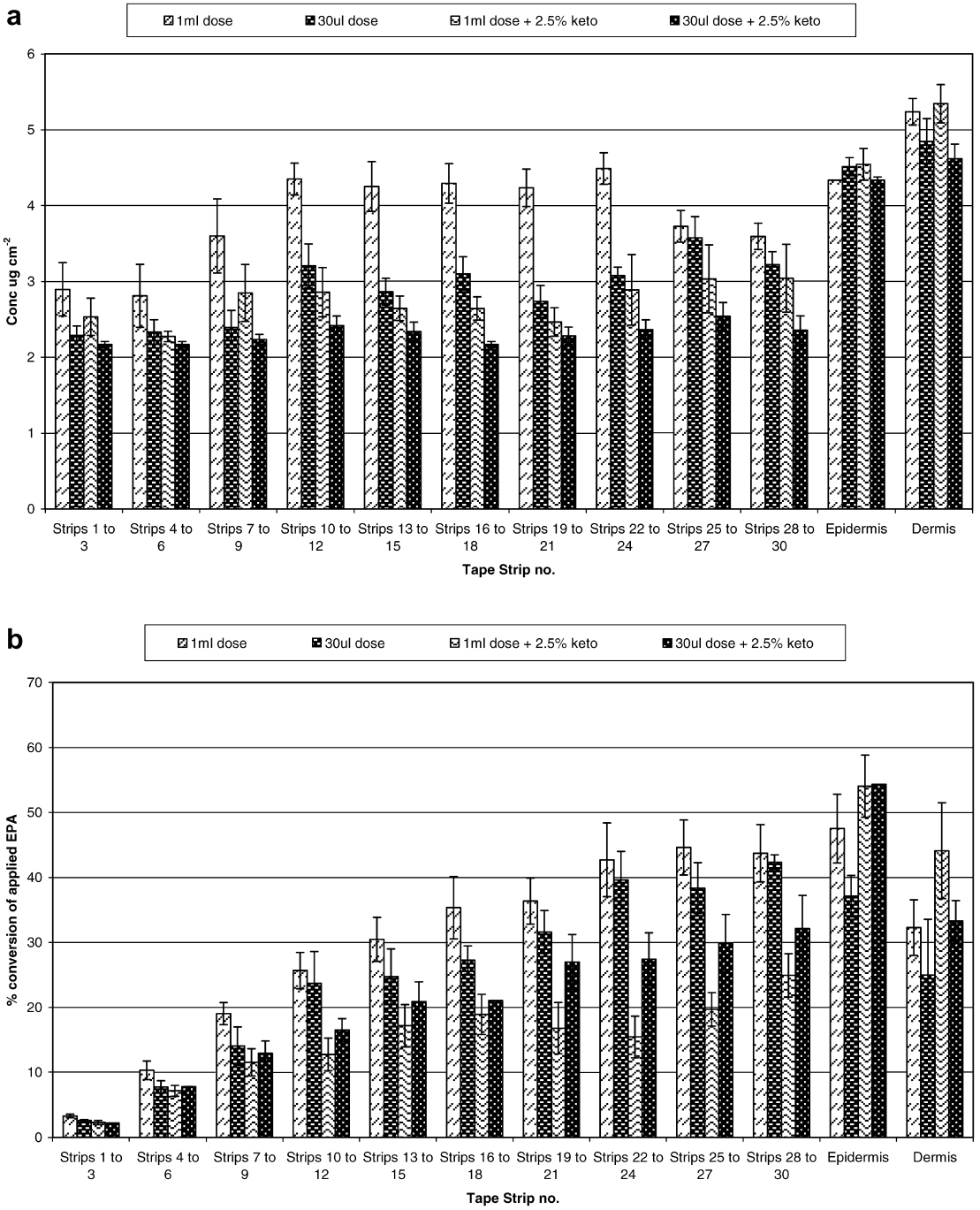

Fig. 5. (a) Concentration of 15-HEPE as a function of depth. (b) Concentration of 15-HEPE as a percentage of EPA at specific depth, growth media-sustained only (±SEM).

As expected the greatest formation of 15-HEPE was seen with 1 ml of fish oil. The change to a finite dose resulted in less metabolite being produced, again as expected. The addition of ketoprofen into the formulation resulted in an even lower concentration of metabolite being produced. As only ketoprofen, fish oil and BHA (fixed at 0.05% wt/wt) were present, this reduction in metabolism of EPA from fish oil must have been due to the ketoprofen as BHA was present in all formulations. The slight increase in the overall production of 15-HEPE as the tape strip number increased was due to the proximity of the EPA to the highly metabolically active basal layer of the epidermis. It also appears that there was a reservoir effect in the remainder of the epidermis after strip number 30 and a higher proportion within the dermis, implying that permeation of 15-HEPE through the epidermis into the dermis occurred. It is possible that some metabolite was generated in the dermis, although it is more likely that 15-HEPE was accumulating at the skin/receptor phase interface.

The production of 15-HEPE was clearly dependent on the concentration of EPA within the skin. Fig. 5b illustrates the concentration of 15-HEPE as a percentage of the EPA present at that particular point within the skin. It is, in effect, the percentage of EPA converted to 15-HEPE at that point within the skin. The shape of the profile corresponds well with the activity of the layers of the epidermis. A greater conversion of EPA to 15-HEPE is seen as the proximity to the highly metabolic basal layer of the skin is increased. The presence of ketoprofen was also seen to exhibit an inhibitory effect upon the metabolism of EPA to 15-HEPE up to the remainder of the epidermis and the dermis. This difference between the upper layers of the epidermis and remainder of the skin may be explained by the possible penetration and accumulation of 15-HEPE within the lower layers of the skin. The inhibited permeation of 15-HEPE into the receptor phase due to the unfavourable sink conditions may result in a reservoir of the compound within the dermis and remaining epidermis. However, the action of ketoprofen in the metabolically

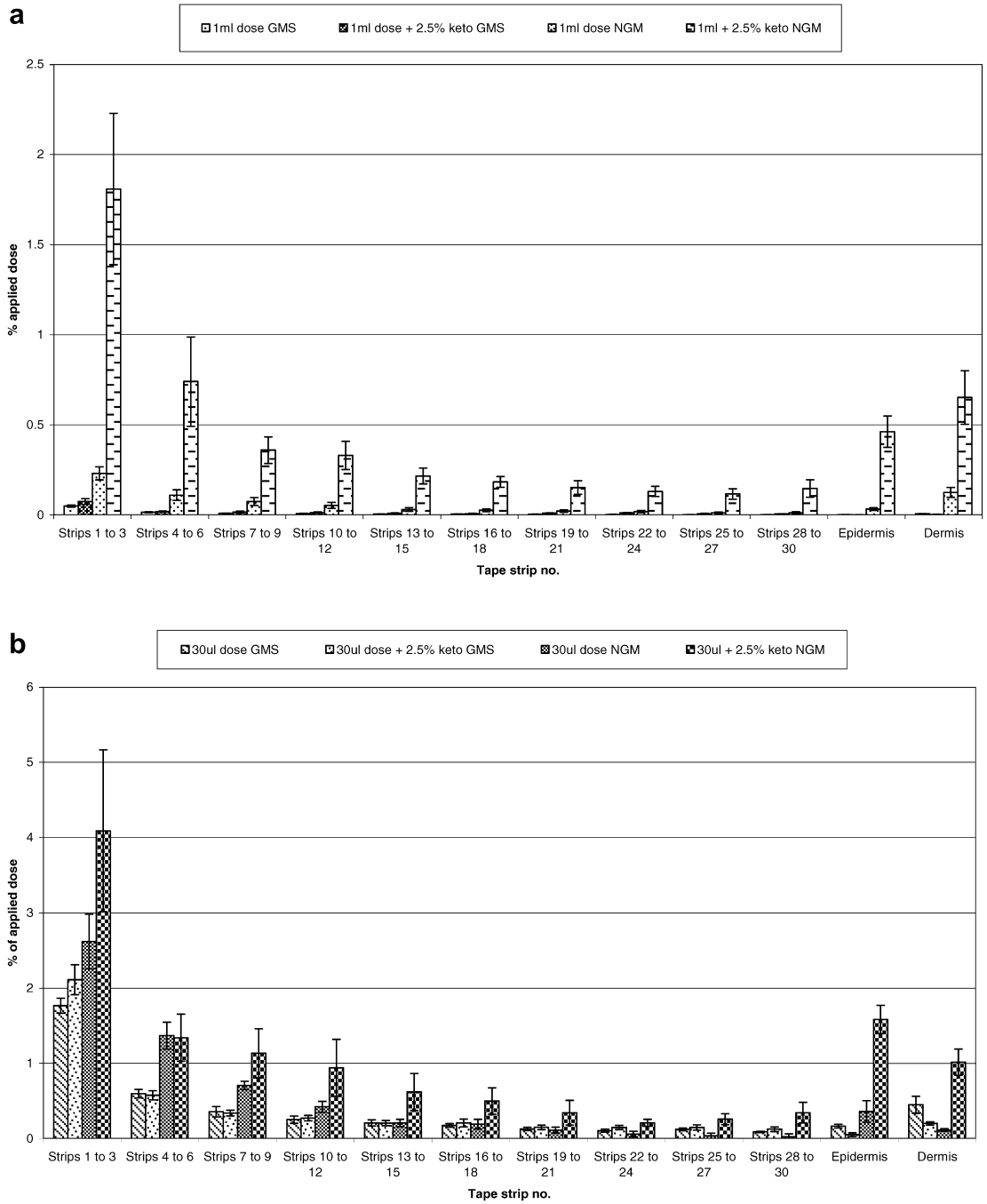

Fig. 6. (a) Comparison of percentage of applied dose of 1 ml formulations. (b) Comparison of percentage of applied dose of 30 μl formulations, growth media-sustained and non-growth media (±SEM).

active layers of the epidermis is significant, especially when comparing infinite doses of the formulation. As ketoprofen is known to act on the COX enzymes and not LOX this is a surprising inference when considering the reduction seen in concentration of LOX produced 15-HEPE. It is likely that some degree of complexation takes place between EPA and ketoprofen leading to less free EPA available to be metabolised. 15-HEPE does not account for all the metabolised EPA however as other catabolic enzymes such as COX are present and will also be metabolising EPA. The action of the formulations containing both EPA and ketoprofen upon epidermal enzymes has also been examined and is awaiting publication [17].

Also evident during the penetration process was plateauing in the rate of metabolism. After the first three strips the concentrations of 15-HEPE for all formulation appear to have become similar and are not significantly different ($p > 0.10$ in all comparisons) indicating that the concentrations of EPA present have saturated the 15-LOX metabolic pathway. Once this saturation has been reached, the penetration of EPA can exceed the metabolism and a constant concentration of metabolite and EPA is seen throughout the layers of the skin. Such flooding of the particular metabolic pathway, COX or LOX, permits the penetration and subsequent permeation of further molecules of EPA in an unconverted state. The concentration of ketoprofen present in the formulations plays an important role in flooding the COX pathway, as if the pathway is competitively inhibited by ketoprofen, this creates the opportunity for non-metabolised EPA permeation. However, if ketoprofen is inhibiting the action of COX then more EPA is left in an unaltered state upon which the LOX enzymes can act. This should then cause an increase in 15-HEPE production, but this was not seen to be the case. If the metabolism of COX is hindered and the 15-LOX pathway becomes saturated, more EPA is left free to penetrate. This would explain greater overall permeation but not the influence of ketoprofen on the metabolism by 15-LOX. An additional explanation for the enhancing ability of ketoprofen is therefore required. 15-LOX is just one enzyme prevalent in the epidermis and it may be the case that other enzymes are just as important in the metabolism of EPA.

A possible complexation interaction between ketoprofen and EPA could go some way to explaining the result [16]. This complexation could cause a drag effect by ketoprofen on EPA allowing greater permeation. The co-permeation of drugs and of drug/solvent complexes is a phenomenon currently under scrutiny [18] and these data could be another example of such an occurrence.

It is not appropriate to compare finite and infinite dosing, as the thermodynamic effects are different, introducing an extra factor in penetration and leading to iniquitous comparisons being made. Fig. 6a shows the comparison of the 1-ml doses of both formulations with both receptor phase solutions. Both non-growth media results show an increased percentage of EPA. It is evident that the presence of ketoprofen is having an effect on the penetration of EPA, be it in the growth media-sustained samples as explained or in the non-growth media samples. This alludes to the possibility of another mechanism in operation within the formulation that enables ketoprofen to enhance the permeation of EPA. This phenomenon is examined in depth in other publications [16,17].

The data from the finite doses are compared in Fig. 6b. The finite dose comparison shows a logical pattern of permeation. The lowest permeation was seen with the 30-μl dose without ketoprofen with the viable, metabolically active, growth media-sustained skin. When ketoprofen was added inhibition of metabolism by 15-LOX was increased permitting more EPA to permeate unchanged. With the non-growth media skin, a greater concentration of EPA permeated as the skin was not viable. The presence of ketoprofen once again enhanced the permeation of EPA as in the 1 ml doses.

4. Conclusions

Several conclusions can be drawn from this investigation. Firstly, it has been shown that metabolism within the skin (in this case via 15-LOX) is unfavourable to the transcutaneous permeation of EPA. The ability of ketoprofen to enhance the penetration of EPA through the skin does negate this to a certain degree, but it must still be considered when designing further EPA permeation experiments. However, metabolism of EPA in the transcutaneous treatment of arthritis does not detract from the system, as 15-HEPE has also been shown to be an effective anti-inflammatory agent.

Evident within this work is the importance of ketoprofen for enhanced EPA permeation in both growth media sustained skin and growth media absent skin. The data from this study have suggested a further explanation for the enhancement demonstrated by Thomas and Heard [8]. The presence of ketoprofen in the formulation appears to decrease the 15-LOX metabolism of EPA and resulting conversion to its metabolite, 15-HEPE.

The apparent link between ketoprofen, enzyme pathways and transdermal penetration of EPA appears to be only part of the solution as even in non-viable skin ketoprofen has a beneficial enhancing effect.

The third conclusion is that the choice of receptor phase solution in skin permeation and specifically skin metabolism studies is a crucial aspect when designing and carrying out such an experiment. As stated in Haberland et al. [19] the slightest change of receptor phase constituents can have a drastic effect on the viability of the skin cells [19]. This study demonstrates that cetrimide, a widely used receptor phase, is not a good choice for this category of work and that a growth medium should be used in the majority of skin diffusion experiments to try and mimic in vivo conditions as closely as possible. Nevertheless, this issue must be balanced alongside the necessity to provide sink conditions in skin experimentation.

References

[1] J. Belch, Fish oil and rheumatoid arthritis: does a herring a day keep the rheumatologists away? Annals of the Rheumatic Diseases 49 (1996) 71–72.

[2] A.C. Williams, Topical and transdermal drug delivery, Pharmaceutical Press, London, 2004.

[3] C.M. Heard, S.J. Gallagher, J.L. Harwood, P.B. Maguire, The in vitro delivery of NSAIDs across the skin was in proportion to the delivery of essential fatty acids in the vehicle: evidence that solutes permeate skin associated with their solvation cages? International Journal of Pharmaceutics 261 (2003) 165–169.

[4] C.L. Curtis, S. G Rees, J. Cramp, C.R. Flannery, et al., Effects of n-3 fatty acids on cartilage metabolism, Proceedings of the Nutrition Society 61 (3) (2002) 381–389.

[5] C. Puglia, S. Tropea, L. Rizza, N.A. Santagati, F. Bonina, In vitro percutaneous absorption studies and in vivo evaluation of anti-inflammatory activity of essential fatty acids (EFA) from fish oil extracts, International Journal of Pharmaceutics 299 (2005) 41–48.

[6] D.H. Nugteren, E. Christ-Hazelhof, A. van der Beek, U.M.T. Houtsmuller, Metabolism of linoleic acid and other essential fatty acids in the epidermis of the rat, Biochimica et Biophysica Acta 834 (1995) 429–436.

[7] A.P. Simopoulos, Omega-3-fatty acids in inflammation and autoimmune diseases, Journal of the American College of Nutrition 21 (2002) 495–505.

[8] C.P. Thomas, C.M. Heard, In vitro transcutaneous delivery of ketoprofen and polyunsaturated fatty acids from a fish oil vehicle incorporating 1,8-cineole, Drug Delivery 12 (2005) 7–14.

[9] H. Tanojo, J. A Bouwstra, H.E. Junginger, H.E. Bodde, In vitro human skin barrier modulation by fatty acids: skin permeation and thermal analysis studies, Pharmaceutical Research 14 (1) (1997) 42–49.

[10] C.C. Miller, V.A. Ziboh, Human epidermis transforms eicosapentaenoic acid to 15-hydroxy-5,8,11,13,17-eicosapentaenoic acid – a potent inhibitor of 5-lipoxygenase, Journal of the American Oil Chemists Society 65 (4) (1988) 474.

[11] C.C. Miller, W. Tang, V. A Ziboh, M.P. Fletcher, Dietary supplementation with ethyl-ester concentrates of fish oil (n-3) and borage oil (n-6) polyunsaturated fatty acids induces epidermal generation of local putative anti-inflammatory metabolites, Journal of Investigative Dermatology 96 (1) (1991) 98–103.

[12] K. Vang, V.A. Ziboh, 15-lipoxygenase metabolites of γ-linolenic acid/eicosapentaenoic acid suppress growth and arachidonic acid metabolism in human prostatic adenocarcinoma cells: possible implications of dietary fatty acids, Prostaglandins Leukotrienes And Essential Fatty Acids 72 (2005) 363–372.

[13] H. Mukthtar, Cutaneous Metabolism During Percutaneous Absorption in Pharmacology of the Skin, CRC Press, London, 1992, pp. 111–126.

[14] U. Jacobi, M. Kaiser, H. Richter, H. Audring, W. Sterry, L. Lademann, The number of stratum corneum cell layers correlates with the pseudo-absorption of the corneocytes, Skin Pharmacology and Physiology 18 (2005) 175–179.

[15] C.M. Heard, B.V. Monk, A.J. Modley, Binding of primaquine to epidermal membranes and keratin, International Journal of Pharmaceutics 25 (1–2) (2003) 237–244.

[16] C.P. Thomas, J. Platts, T. Tatchell, C.M. Heard, Probing the skin permeation of fish oil/EPA and ketoprofen 1. NMR spectroscopy and molecular modelling, in preparation.

[17] C.P. Thomas, Z. Davison, C.M. Heard, Probing the skin permeation of fish oil/EPA and ketoprofen 3. Influence of fish oil/ketoprofen on epidermal COX-2 and LOX, in preparation.

[18] C.M. Heard, D. Kung, C.P. Thomas, Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect, International Journal of Pharmaceutics 321 (2006) 167–170.

[19] A. Haberland, S. Schreiber, C. Santos Maia, M. K Rübbelke, M. Schaller, H.C. Korting, B. Kleuser, I. Schimke, M. Schäfer-Korting, The impact of skin viability on drug metabolism and permeation – BSA toxicity on primary keratinocytes, Toxicology In Vitro 20 (3) (2006) 347–354.