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Note

The in vitro delivery of NSAIDs across skin was in proportion to the delivery of essential fatty acids in the vehicle—evidence that solutes permeate skin associated with their solvation cages?

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Abstract

As part of our investigations into novel dual action topical anti-arthritis systems, the permeation of ibuprofen or ketoprofen plus eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were determined from a fish oil vehicle across pig ear skin in vitro. The steady state fluxes of ibuprofen and ketoprofen were $9.17 \pm 1.98\ \mu g\ cm^{-2}\ h^{-1}$ and $6.12 \pm 2.39\ \mu g\ cm^{-2}\ h^{-1}$, respectively. At 24 h, 5.7 $\mu g\ cm^{-2}$ EPA and 3.1 $\mu g\ cm^{-2}$ DHA permeated when the solute was ibuprofen; 1.4 $\mu g\ cm^{-2}$ EPA and 1.0 $\mu g\ cm^{-2}$ DHA when ketoprofen was the solute. At 12 h, the ketoprofen/ibuprofen ratio of the moles permeated was 0.27, the ratio of EPA permeated simultaneously with ketoprofen and ibuprofen was 0.22 and the ratio of DHA permeated simultaneously with ketoprofen and ibuprofen was 0.24. We believe this is the first time that simultaneous permeation across skin of a solute and its vehicle has been determined purposefully. The data successfully demonstrated that simultaneous permeation of NSAIDs and essential fatty acids, EPA and DHA from a formulation containing fish oil is feasible. In addition, for both NSAIDs, the relative rates of permeation of EPA and DHA, were in proportion to their levels in the fish oil and the permeation rate of either fatty acid was higher when the permeation rate of the solute was greater. This suggested that the greater the rate of permeation of the NSAID, the greater the rate of permeation of the vehicle, and that a solute permeates skin complete with its vehicular solvation cage. This apparent relationship between solute and vehicle fluxes may be of more widespread significance to skin permeation experimentation.

Keywords: Arthritis; Ibuprofen; Ketoprofen; Fish oil; Transcutaneous delivery; *n*−3 fatty acids; Vehicle

NSAIDs play an important role in the management of rheumatoid and osteo arthritis (Wood, 1999), with ibuprofen and ketoprofen frequently indicated to control pain and inflammation. However, NSAIDs are associated with gastrointestinal upset and because the medicines are usually taken orally, their effects are generalised. The efficacy of topical preparations can be limited (Moore et al., 1998) because of poor rates of transcutaneous delivery, chiefly attributable to the barrier function of the skin (Hadgraft and Walters, 1994).

A number of studies have demonstrated the benefit of fish oil in arthritis (Lau et al., 1993; Fortin et al., 1995). Fish oil has been found to be a strong inhibitor of the inflammatory consequences of eicosanoids derived from arachidonate (Belch, 1990) which has

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been attributed to a high content of $n-3$ polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are often referred to as 20:5 and 22:6, respectively, in that EPA is a 20 hydrocarbon chain with five double bonds whereas DHA is a 22 hydrocarbon chain with six double bonds. Both are metabolised to the weakly inflammatory 3-series prostaglandins and 5-series leukotrienes and compete with the inflammatory eicosanoids derived from $n-6$ fatty acids such as arachidonate, which are strongly inflammatory. More recent studies provided a molecular basis for why $n-3$ polyunsaturated fatty acid supplementation could be of benefit in the relief of many of the important symptoms found in arthritis (Curtis et al., 2000). However, dietary supplementation with fish oil is inherently inefficient, again because of its wide distribution throughout the body and the relatively low blood supply to joints.

The primary aim of this work was to examine the plausibility of delivering simultaneously an NSAID plus the essential $n-3$ polyunsaturated acids EPA and DHA from a topically applied dose containing the drug and fish oil. Saturated solutions of ibuprofen and ketoprofen (Sigma, Poole, UK) were prepared at 32 °C in fish oil purchased from a local store (Boots the Chemist, UK, batch BA55). To model human transcutaneous delivery, freshly excised full thickness pig ear skin membranes were used (Dick and Scott, 1992). The ears were cleaned under running water, the hair shaved using an electric razor and full thickness dorsal skin removed from the underlying cartilage using a scalpel, before being cut into approximately 2 cm^2 specimens and mounted equally distributed between the treatments in all-glasss Franz-type cells, with nominal diffusional area of 0.78 cm^2 and receptor volume of 3.5 ml. The receptor phase was degassed 30 mg ml^{-1} aqueous cetrimide (Heard et al., 2002), which provided an alcohol-free (Saunders and Pugh, 2002) sink for both the NSAID and the oil. Butylated hydroxy anisole (0.05% w/v) was added to the donor and receptor phases to inhibit oxidation of the polyunsaturated fatty acids. The receptor compartments were filled and the cells placed on a magnetic stirring plate set-up in a water bath set at 37 °C, thereby maintaining skin surface temperature of approximately 32 °C. After 15 min, nine cells were dosed with 200 μl of saturated solution of ketoprofen or ibuprofen and the top of the donor chamber occluded. In addition, two control cells were included which were dosed with the C8-9 diacylglycerol Miglyol 840N (Condea, Germany). Whole receptor phases were removed at 3, 6, 12, 24 and 48 h and replaced with fresh solution. Four hundred microlitre of each sample was retained for HPLC analysis of the NSAID and the remainder retained for GC analysis of the EPA and DHA. Samples were stored at −20 °C prior to analysis.

Ibuprofen and ketoprofen were determined using an Agilent 1100 automated HPLC system fitted with a Kingsorb 5 μ C18 250 mm × 4.6 mm column (Phenomenex, Macclesfield, UK) and an ultraviolet detector, pump flow rate of 1 ml min^{-1}. For ibuprofen, the mobile phase was methanol (85%), water (15%) and trifluoroacetic acid (0.1%) and detection at 230 nm. The mobile phase for ketoprofen was acetonitrile (55%), 0.01 M potassium phosphate adjusted to pH 1.5 with orthophosphoric acid (45%) and detection at 258 nm. Fatty acid methyl esters of EPA and DHA were prepared by acid-catalysed methanolysis and then analysed using an Autosystem XL gas chromatograph (Perkin-Elmer Instruments, Beaconsfield, UK), fitted with a glass column (1.5 m × 3.0 mm) packed with 10% SP-2330 on 100/120 mesh Supelcoport (Supelco, Sigma, Poole, UK) following the general method described by Curtis et al. (2000). Steady state flux was determined from the least squares regression through the linear portion of the cumulative permeation curves, lag time found by extrapolation of the regression to the x axis.

Permeation data for ibuprofen and ketoprofen are summarised in Table 1. The mean flux for ibuprofen was 9.17 μg cm^{-2} h^{-1} with a standard error of 1.98. Ketoprofen had a mean flux of 6.23 μg cm^{-2} h^{-1} with a standard error of 2.39. The steady state flux of ibuprofen was thus 1.5 times that of ketoprofen, although both NSAIDs were administered as saturated solutions hence thermodynamic activity $= 1$. Also, the lag time of ketoprofen was approximately double that of ibuprofen. However, as ibuprofen was considerably more soluble than ketoprofen in the fish oil (Table 1), the results are more in keeping with Fickian laws of diffusion. Ceschel et al. (2002) examined the permeation of ketoprofen from saturated solutions in pH 6.5 buffer plus various co-solvents and concluded that diffusion constant, D, did not change markedly, although partition coefficient, P, was greatly influenced.

Table 1
Summary of the solubility, flux, lag time and K_p data for ibuprofen and ketoprofen from fish oil vehicle ($n = 9$, ±S.E.M.)

NSAID	Solubility (mg ml^{-1})	Steady state flux (μg cm^{-2} h^{-1})	Lag time (h)	$K_p \times 10^{-5}$ (cm h^{-1})
Ibuprofen	309.6 ± 25.8	9.17 ± 1.98	3.1	2.96
Ketoprofen	72.7 ± 3.4	6.23 ± 2.4	5.8	8.6

However, potential diffusion and permeation of the vehicle itself was overlooked.

Control experiments, in which the skin was dosed with Miglyol 840 (saturated C8-9 diacylglycerol), showed no changes in the fatty acid composition of the skin or presence of EPA and DHA in the receptor phase after 48 h from the skin. However, the cumulative permeation profiles of EPA and DHA shown in Fig. 1 clearly demonstrate that these two essential fatty acids permeated the skin when dosed with fish oil (and NSAID). Some plateauing was observed, probably due to saturation of the aqueous receptor phase with permeated oil. The permeation rate of EPA was consistently greater than DHA reflecting their relative proportions in the fish oil: EPA = 42%; DHA = 27% (balance mainly saturated and arachidonic acids). In the oil, both would be at equal thermodynamic activity, so the results are again generally in keeping with Fickian transport laws. At 24 h, 5.7 μg cm^{-2} EPA and 3.1 μg cm^{-2} DHA permeated the pig ear skin when the solute was ibuprofen; 1.4 μg cm^{-2} EPA and 1.0 μg cm^{-2} DHA when ketoprofen was the solute. These figures represent just 0.008, 0.007, 0.002 and 0.002%, respectively, of the applied doses (mass of 200 ml fish oil = 0.178 g) and are a consequence of the 'infinite' dose applied. The potential of EPA and DHA free acids to act as skin penetration enhancers has been demonstrated in experimental animals (Loftsson et al., 1995), although we are not aware of any reports of the deliberate delivery of these compounds across skin. Tanojo et al. (1997) reported that polyunsaturated fatty acids—linoleic (LA), linolenic (ALA) and arachidonic acids—enhance *p*-aminobenzoic acid permeation stronger than mono-unsaturates, but

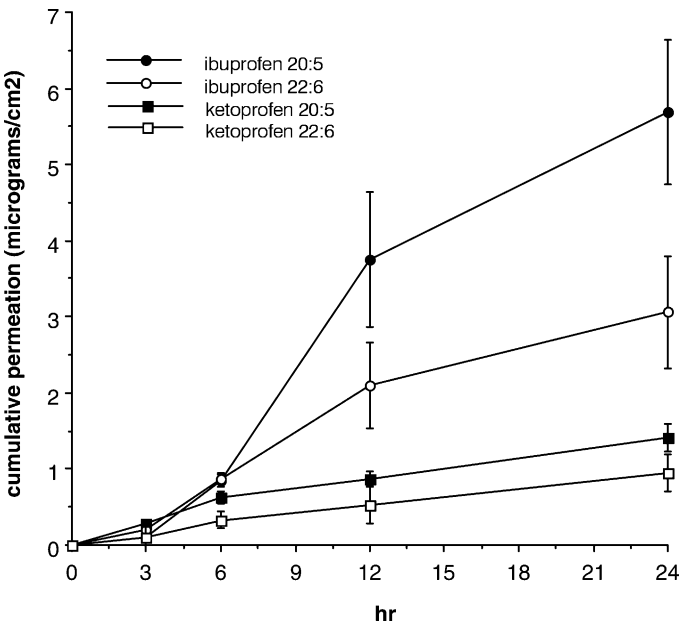

Fig. 1. Cumulative permeation profiles for the delivery of *n*−3 fatty acids EPA (20:5) and DHA (22:6) across excised pig skin from a fish oil vehicle co-permeated either ibuprofen or ketoprofen ($n = 9$, ±S.E.M.).

Table 2
Ratios of molar masses permeated (cm^{-2}) at 12 h as a function of solute

Permeants	Ketoprofen	Ibuprofen	EPA (20:5) ketoprofen	EPA (20:5) ibuprofen	DHA (22:6) ketoprofen	DHA (22:6) ibuprofen
$\mu g\,cm^{-2}$	25.0	75.0	0.85	3.8	0.50	2.1
$mol\,cm^{-2}$	9.8×10^{-8}	3.6×10^{-7}	2.8×10^{-9}	1.25×10^{-8}	1.52×10^{-9}	6.4×10^{-9}
Ratio (*K*/*I*)	0.27		0.22		0.24	

additional double bonds did not further increase the degree of enhancement. But again their interest was in fatty acids, rather than triacylglycerols.

An interesting observation in the current data is that both EPA and DHA permeated at a faster rate simultaneously with the solute that permeated faster (i.e. ibuprofen). This suggested an unexpected link between permeation of the solute and the vehicle in which it was dissolved, which only came to light as in our experiments both solute and vehicle were determined. We believe this is the first time the vehicle has been considered as a permeant and, therefore, its permeation across skin determined purposefully. Many skin permeation experiments involve permeant molecules dissolved in liquid or semi solid vehicles. As such, each molecule of permeant will be associated with a cage of vehicle molecules. Although it is well known that some vehicles increase the partition coefficient of the permeant into the stratum corneum, permeant/vehicle association thereafter appears from the literature to have been completely overlooked.

Table 2 shows the molar ratios of masses permeated (cm^{-2}) at 12 h (steady state, non-saturation of donor phase) as a function of solute. The ratio of ketoprofen to ibuprofen permeated was 0.24. The same ratio was also observed for both EPA and DHA. The data suggests that when a solute molecule traverses the skin from a liquid (and perhaps semi-solid) vehicle it does so still whilst remaining at least partially associated with its solvation cage from the applied dose. Consequently, the permeation rates observed pertain not simply to the permeant of interest, but of the *overall* solvated complex. Most typical skin permeation experimental set-ups would be incapable of such observations without the ability to solvate both solute and vehicle in the receptor phase, in addition to a desire to determine the permeation of the vehicle.

Further support for such effects, which may help explain anomalies in the development and use of theoretical predictive methods of skin permeation, exists in a number of guises. For example, the 'ultradeformable' liposomes as described by Cevc et al. (2002), postulate large complexes that allegedly permeate skin intact. In addition, reverse iontophoresis of glucose relies upon electro-osmosis of the water solvent cage surrounding the neutral analyte, albeit under the influence of an applied potential, to transport it across the skin for subsequent determination. It also seems reasonable to assume that the thermodynamic favourability that that allowed the solute to dissolve in the vehicle in the first place would not necessarily be altered through interactions between the solute/solvent cage and components of the skin.

The current observations may have important implications for other work in that the potential permeation of the vehicle and/or other excipients across skin may be of significance not only in interpreting permeation data, but also in terms of potentially significant doses of vehicle entering the system.

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