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In-vitro transcutaneous delivery of ketoprofen and polyunsaturated fatty acids from a pluronic lecithin organogel vehicle containing fish oil

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Abstract

This work explored the use of pluronic lecithin organogel (PLO) as a base for the delivery of bioactive polyunsaturated fatty acids from fish oil, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and ketoprofen. PLO was adapted to contain fish oil, ketoprofen, or both, and 1,8-cineole as penetration enhancer, and used to determine the in-vitro permeation from infinite and finite dosing protocols across full thickness porcine skin. Oruvail gel (2.5% ketoprofen) was included for comparison. No EPA or DHA was found to permeate skin when applied as an infinite dose. From multiple finite doses, small amount (max. 0.22%) of fish oil were found to permeate the skin. This indicates retention of fish oil within the gel matrix and that the viable domain of full thickness skin was a significant barrier. Greater amounts of EPA and DHA were delivered in the presence of ketoprofen indicating co-transport resulting from selective complexation, although no enhancement was observed using 1,8-cineole. Unlike EPA and DHA, substantial amounts of ketoprofen permeated when applied as infinite doses. Oruvail, a Carbopol 940-based hydrogel containing 2.5% ketoprofen and ethanol, delivered the greatest amount, although similar to the PLO gel containing 5% ketoprofen. The addition of propylene glycol enhanced permeation, although the presence of fish oil in the PLO gel inhibited ketoprofen permeation. When applied as multiple finite doses a maximum of 76 μm cm^{-2} (1.12%) was delivered, which was reduced by the presence of 1,8-cineole. Greater permeation was again observed with Oruvail by a factor of two and with half the ketoprofen dose. To conclude, a PLO-based gel is capable of delivering EPA and DHA via a repeat finite dosing regimen, although there is evidence for the retention of these very lipophilic molecules within the gel matrix. Although to a lesser extent than EPA and DHA, ketoprofen was also substantially retained, as exemplified by the superior delivery rates from Oruvail. Finally, this work has highlighted the importance of using an appropriate topical dosing method to match the intended use of a product.

Introduction

The n-3 long-chain fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are recognised for their anti-inflammatory properties (Calder 2001). This is based upon their ability to compete with arachidonic acid as a substrate for cyclo-oxygenase and lipoxygenase, which results in the production of less potent eicosanoids, prostaglandins and leukotrienes (Ziboh et al 2000). Fish oil is a major source of EPA and DHA and has a long history of demonstrable health benefits, both anecdotal and scientific (Curtis et al 2004).

Dietary supplementation with fish oil has also been shown to reduce prostaglandin E2 levels within the skin, thereby improving UVB erythemal sensitivity (Rhodes et al 1994), although the beneficial effects of dietary supplementation with fish oil is more frequently associated with arthritis (Remans et al 2004). However, oral dosing is inherently ineffective, partly as the target areas (arthritic joints) are poorly served by the systemic circulation. For example, up to 18 g of fish oil is required to obtain any discernible beneficial effects (Cleland et al 1988). We are thus investigating the plausibility of topically applied alternatives, where the oil and non-steroidal anti-inflammatory drug (NSAID) are delivered locally in the vicinity where it is required. Local

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bioavailability also avoids first-pass hepatic metabolism and side-effects. Delivery of polyunsaturated fatty acids (PUFAs) from fish oils across skin has previously been demonstrated from liquid formulations, where the co-permeation of an NSAID (e.g. ketoprofen) can work synergistically with the EPA and DHA (Heard et al 2003; Puglia et al 2005; Thomas & Heard 2005).

In this work we examined the in-vitro transcutaneous delivery of ketoprofen and EPA/DHA from fish oil using gels based upon pluronic lecithin organogel (PLO). Pluronic gel is a poloxamer made of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide). The most common form of pluronic gel used in the compounding market today is Pluronic F-127 (PF-127), which has unique physiologic characteristics – as it warms it becomes a thermoreversible solid (gel) and as it cools it becomes a liquid. Due to the unique physiologic nature of pluronic gel, adding certain enhancers like lecithin, derived from the soy plant, improves the effectiveness of the pluronic gel. These are of interest, particularly within veterinary applications, as they are purported to possess superior delivery properties to other gel formulations (Vitoria et al 1999). More specifically, it was hypothesised that the oily component of a typical PLO (isopropyl palmitate) could be replaced by fish oil, principally triacylglycerols and PUFA-esters, maintaining the efficacy of the PLO vehicle (Barichello et al 1999; Escobar-Chavez et al 2005) while simultaneously delivering EPA and DHA (and ketoprofen) across skin. Consequently, both infinite and finite dosing protocols were employed with and without penetration enhancer (Williams & Barry 2004), where the finite dosing involved multiple re-applications to represent a plausible dosing regimen (Bowen & Heard 2006). For comparative purposes this study also examined a PLO gel containing propylene glycol and a commercial preparation of ketoprofen, Oruvail.

Materials and Methods

Materials

Fish oil capsules (1000 mg, Boots Super Strength, batch number 100673; stated composition, 33% EPA, 21% DHA) and Oruvail 2.5% w/w ketoprofen gel (batch number IP8063) were obtained from a local store. Pluronic F-127, ketoprofen, butylated hydroxyanisole (BHA) 1,8-cineole, Cetrimide BP, EPA and DHA were obtained from Sigma-Aldrich Company Ltd (Poole, UK). Nylon membranes (0.2 and 0.45 micron pore) were obtained from Phenomenex (Macclesfield, UK). Methanol, petroleum ether and lecithin were obtained from Fisher Chemicals (Loughborough, UK). All other reagents were of analytical grade or equivalent. Freshly excised pig ears were obtained from a local abattoir before steam cleaning.

Preparation of gel formulations

Eight PLO-based gels were prepared (Table 1) by adapting a previously published method (Barichello et al 1999). Ethanol was used as a solvent for ketoprofen and fish oil instead of isopropyl palmitate. The required amounts of the antioxidant BHA and fish oil were measured and vortex mixed. Lecithin was measured into a glass bottle and the required amount of ethanol added. Ketoprofen was incorporated to provide 2.5 or 5% w/w drug in the completed formulation. The required amount of Pluronic F-127 was measured and made up to 20% w/w with cold de-ionised water and stored overnight at 2–4°C to allow complete dissolution of the polymer. The liquid phase and required amount of gel were thoroughly mixed to provide the final product. A blank gel was prepared similarly, along with gels incorporating propylene glycol or 5% w/w 1,8-cineole as penetration enhancer (Yamane et al 1995; Thomas & Heard 2005).

Table 1 Formulae of PLO-based gels examined in this work

Gel	Composition (%)							
	I Fish oil	II Fish oil Cineole	III Fish oil Ketoprofen	IV Fish oil Ketoprofen Cineole	V Ketoprofen 2.5%	VI Ketoprofen 5%	VII Ketoprofen 2.5% Propylene glycol	VIII Blank
Lecithin	10	10	10	10	10	10	10	10
Ethanol	10	10	10	10	10	10	10	10
Ketoprofen	—	—	5	5	2.5	5	2.5	—
Fish oil	10	10	10	10	—	—	—	—
BHA	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
1,8 Cineole	—	5	—	5	—	—	—	—
20% w/w PF-127 soln	69.5	64.5	64.5	59.5	77	74.5	67	79.5
Propylene glycol	—	—	—	—	—	—	10	—

Receptor solution

A 30 mg mL^{-1} cetrimide solution was previously shown to be a suitable receptor phase (Heard et al 2003; Thomas & Heard 2005). Cetrimide is anti-microbial and solubilises fish oil and ketoprofen, thus providing good sink conditions. It has also been shown to perform similarly to aqueous alcohol as a receptor phase (Heard et al 2002). The required amount of cetrimide and the antioxidant BHA (0.05% w/w of cetrimide) were incorporated with sufficient distilled water. Cetrimide was dissolved on a magnetic stirring plate and the solution degassed by vacuum filtering through a 0.45 μm cellulose membrane to remove dissolved air. In this receptor phase solution ketoprofen was previously found to be freely soluble and the solubility of fish oil was 5.3 ± 0.3 mg mL^{-1} (1.73 ± 0.01 mg EPA mL^{-1}).

In-vitro transcutaneous delivery

In-vitro skin permeation evaluation, involving Franz-type diffusion cells incorporating excised skin, has previously demonstrated good in-vivo/in-vitro correlation (Valiveti et al 2005) and porcine ear skin is believed to be a good model for human skin permeability (Simon & Maibach 2000). Five porcine ears were cleaned under running water and the full thickness skin removed from the dorsal side by blunt dissection. Sections, 2×2 cm, were distributed among the formulation types to eliminate donor variability, before being placed with the stratum corneum uppermost on pre-greased flanges of the receptor compartment. The donor compartments or 1-cm diameter washers (for finite dosing experiment) were placed on top and pinch-clamped in place, and micro magnetic stirring bars included. A 10-mL syringe with attached tubing was used to fill the receptor compartments with pre-equilibrated receptor phase before being placed on a magnetic stirring plate (Variomag, Daytona Beach, FL, USA) in a water bath maintained at 37°C to provide a skin surface temperature of 32°C via heat dissipation. After 10 min, the cells were dosed with either 1 mL gel (infinite) or 30 μL (27 mg) of gel at 0, 6, 12, 24 and 36 h (multiple finite) – the latter by gently massaging into the skin surface using the flattened end of a glass rod with 4 circular motions. Receptor compartments were replenished with new, temperature-equilibrated receptor phase. Sampling arms of cells were occluded, the donor chambers were not. Entire receptor phases were removed at time points of 6, 12, 24, 36 and 48 h using cell-dedicated Pasteur pipettes. Samples of 500 μL were analysed immediately for ketoprofen and the remainder stored at −20°C before EPA and DHA analyses. Six replicates were run on each occasion.

HPLC analysis

Ketoprofen

Ketoprofen was analysed using an Agilent series 1100 HPLC system, fitted with a Kingsorb 5 μm C18 150×4.6 mm column (Phenomenex, Macclesfield, UK), including a Phenomenex Securiguard guard column (Gallagher et al 2003). Detection was by UV at $\lambda = 258$ nm. The mobile phase was degassed acetonitrile (55%) and 0.01 M potassium phosphate in de-ionised water (45%), adjusted to pH 1.5 with phosphoric acid, at a flow rate of 1 mL min^{-1}. The sample injection volume was 20 μL and the run time was 10 min. The retention time of ketoprofen was 6.8 min under these conditions. The limit of detection (LOD) was found to be 0.03 μg mL^{-1}. Standard solutions of ketoprofen in receptor phase at concentrations were determined by HPLC. A standard calibration curve was constructed over the range 1–120 μg mL^{-1}, which provided an r^2 of > 0.99.

EPA and DHA

EPA and DHA contained within the receptor phase samples were firstly converted to fatty acid methyl esters (FAMEs) by acid methanolysis (yield ≥95%). Samples were immersed in liquid nitrogen and freeze-dried overnight to remove water, as it prevents the transmethylation reaction from going into completion. PUFAs were methylated by adding 10 mL of an anhydrous methanol and acid catalyst (2.5% H_2SO_4) solution to samples and heating under reflux at 70°C for 2 h. Solutions were allowed to cool to room temperature and reactions quenched by the addition of 2.5 mL aqueous NaCl (5% w/v). FAMEs were extracted with 3×30 mL petroleum ether (bp 60–80°C) and the ether phase dried under vacuum using a rotary evaporator. Remaining products were dissolved in methanol (1 mL) and transferred to HPLC vials. Nitrogen gas was bubbled through before sealing. Vials were stored under light exclusion before analysis. FAMEs were analysed using a method developed in-house, which was as described above except that the wavelength was 210 nm, the mobile phase was methanol–de-ionised water (95:5) and the run time was 20 min. Under these conditions EPA and DHA eluted at 10.2 and 12.2 min, respectively. The LOD was 0.40 μg mL^{-1} and 0.50 μg mL^{-1} for EPA methyl ester and DHA methyl ester, respectively.

Data analysis

Cumulative ketoprofen, DHA and EPA permeation concentrations (μg cm^{-2} or mol cm^{-2}) were plotted against time, ± standard deviation. Where appropriate, steady-state flux was determined from the gradient of the linear portion of the plots. Statistical analyses were carried out using Instat 3 for Macintosh (GraphPad Software, Inc.), where non-parametric Kruskal–Wallis analysis of variance and Dunn's multiple comparison tests were employed to determine differences between data sets and specific pairs, respectively.

Results and Discussion

In-vitro transcutaneous delivery

Infinite dose

Under infinite dosing conditions, a sufficient amount of formulation is placed upon a skin sample such that

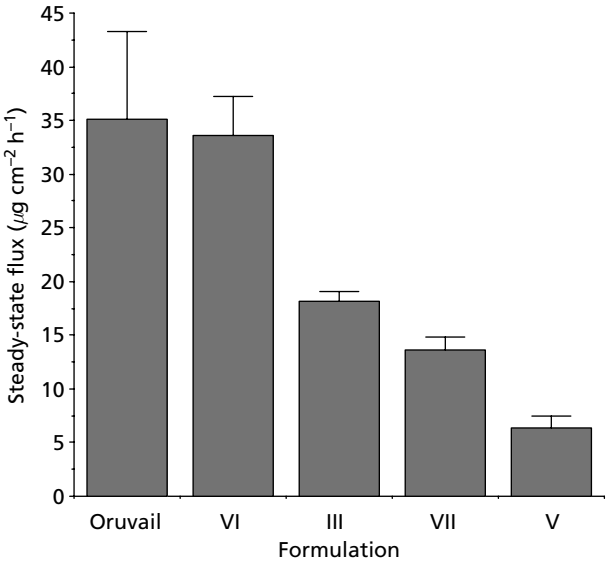

Figure 1 Plot of steady-state flux of ketoprofen across pig ear skin from five formulations applied as infinite dose: Oruvail (2.5% ketoprofen), VI (PLO with 5% ketoprofen, III (PLO with 5% ketoprofen and fish oil), VII (propylene glycol-based PLO with 2.5% ketoprofen), V (PLO with 2.5% ketoprofen). Data are means ± s.d., n = 6.

substantial depletion does not occur. Steady-state kinetics for ketoprofen were observed in each case, indicating no appreciable barrier modulation (e.g. by cetrimide) in the receptor phase. Kruskal–Wallis testing provided a P value of 0.0001 for the steady-state fluxes from formulations III, V, VI, VII and Oruvail. The greatest steady-state flux ($35.15 \pm 8.13\,\mu g\,cm^{-2}\,h^{-1}$) was obtained from Oruvail – a Carbopol 940-based hydrogel containing 2.5% ketoprofen and ethanol (Figure 1). This flux was statistically the same ($P \geq 0.05$) as that achieved for PLO gel VI ($33.57 \pm 3.64\,\mu g\,cm^{-2}\,h^{-1}$) containing twice the amount of ketoprofen, at 5%, but lacking fish oil. From PLO gel III, containing 5% NSAID and 10% fish oil, the flux was $18.17 \pm 0.93\,\mu g\,cm^{-2}\,h^{-1}$ (i.e. halved, although $P > 0.05$). As no EPA or DHA was found to permeate skin, this observation suggests that the presence of the fish oil inhibited ketoprofen permeation, probably as a consequence of the π–π orbital complexation previously observed (Heard et al 2005). The PLO gel VII, containing 2.5% ketoprofen and propylene glycol, provided a ketoprofen flux of $13.64 \pm 1.20\,\mu g\,cm^{-2}\,h^{-1}$, significantly lower than Oruvail ($P < 0.05$), again suggesting a retentive mechanism, despite the established penetration enhancement capability of propylene glycol. Inhibition of ketoprofen permeation by fish oil may be due to competition between micelles for lipoidal domains of the stratum corneum.

Permeation of ketoprofen from gel V (@ 2.5%) was half that from gel VII containing 2.5% ketoprofen and propylene glycol, indicating a degree of enhancement by the propylene glycol and also suggesting that propylene glycol enhanced ketoprofen by the drag effect (Kadir et al 1987; Bowen & Heard 2006). However, if this was the case it is doubtful the micellar nature of the PLO gel was of benefit as a delivery mechanism (Cevc 2004). Moreover, the PLO gel V, equivalent to Oruvail (i.e. both @ 2.5% ketoprofen), provided the lowest flux and was ~ 6-fold lower than Oruvail ($P < 0.001$). From blank gel VIII, none of the analytes were detected after 48 h.

No measurable amount of EPA and DHA permeated when infinite doses of gels were applied to full-thickness pig ear skin in-vitro after 48 h. This was surprising given the positive data reported in earlier papers and would appear to reflect retention within the bulk formulation or at the hydrophilic boundary of the viable epidermis to the diffusion of these very lipophilic permeants.

Finite dosing

This part of the work was restricted to gels II (Fish oil + cineole), III (Fish oil + ketoprofen) and IV (Fish oil + ketoprofen + cineole) and Oruvail. Table 2 shows that, under the dosing regime employed, EPA and DHA were observed in the receptor phase, although steady-state fluxes were not attained. Kruskal–Wallis testing provided a P value of 0.0001. Furthermore, the permeation was at low levels and percentages of the doses applied (max. 0.22%). From each PLO gel containing fish oil, more EPA was delivered than DHA, generally reflecting the relative proportions in fish oil (33% and 21%, respectively). The presence of ketoprofen in Gel III appeared to enhance the permeation of EPA and DHA by some 37% ($P < 0.001$ at both 12 and 24 h). Such an unusual observation has previously been linked to preferential interactions between the π orbitals of an aromatic permeant and the polyunsaturated fatty acids (Heard et al 2005). The presence of the 1,8-cineole appeared not to enhance the permeation of EPA and DHA, as a small reduction was observed ($P \geq 0.05$ at both 12 and 24 h). No earlier publications involving the administration of finite doses of fish oil were found with which to compare the current data.

Under in-use conditions, a small portion of gel would be worked into the skin by a massaging action. Furthermore, the majority of dermatological formulations involve re-application to the same areas as appropriate. In this work a multiple-dosing regime was employed, involving 27 mg of formulation being massaged into the skin at 0, 6, 12, 24 and 36 h (total 135 mg formulation). As no EPA or DHA was found to permeate skin by infinite dosing this indicates that the fish oil was retained within the PLO gel matrix. It also demonstrates that the process of massaging the formulation into the skin is a key event in the transfer of permeant into the stratum corneum, although, as steady-state kinetics were not attained, the dosage regime was unsuccessful in saturating the skin, with the viable epidermis again acting as a barrier to the lipophilic EPA and DHA.

Table 3 reveals that between 12 and 24 h the ratio of DHA to EPA permeating the skin increased from the three formulations containing fish oil. These data suggest either preferential permeation or, as freshly excised skin was utilised, metabolism within the skin. The conversion of EPA by 15-lipoxygenase in skin has been reported from oral doses where the metabolite 15-hydroxyeicosapentaenoic acid (15-HEPE) was found to be a non-potent

Table 2 Transcutaneous delivery across full-thickness pig ear skin of ketoprofen, EPA and DHA from multiple finite doses ($5 \times 27\,mg = 135\,mg$ total) of Gel II, Gel III, Gel IV and Oruvail

Gel/analyte (amount dosed)		Permeated @	12 h	24 h
Gel II	DHA	Mass ($\mu g\,cm^{-2}$)	0.38 ± 0.09	0.91 ± 0.12
(fish oil, cineole)	(2.84 mg/8.66 μmol)	Moles ($nmol\,cm^{-2}$)	1.24 ± 0.30	3.01 ± 0.40
		%, mean	0.013	0.032
	EPA	Mass ($\mu g\,cm^{-2}$)	2.69 ± 0.13	3.65 ± 0.23
	(4.46 mg/14.8 μmol)	Moles ($nmol\,cm^{-2}$)	8.20 ± 0.40	11.1 ± 0.70
		%, mean	0.060	0.082
Gel III	DHA	Mass ($\mu g\,cm^{-2}$)	1.50 ± 0.22	4.44 ± 0.99
(fish oil, ketoprofen)	(2.84 mg/8.66 μmol)	Moles ($nmol\,cm^{-2}$)	4.57 ± 0.67	13.50 ± 3.02
		%, mean	0.053	0.16
	EPA	Mass ($\mu g\,cm^{-2}$)	7.02 ± 1.03	10.03 ± 1.42
	(4.46 mg/14.8 μmol)	Moles ($nmol\,cm^{-2}$)	23.30 ± 3.41	33.11 ± 4.70
		%, mean	0.16	0.22
	Ketoprofen	Mass ($\mu g\,cm^{-2}$)	40.0 ± 8.3	75.7 ± 11.0
	(6.75 mg/26.6 μmol)	Moles ($nmol\,cm^{-2}$)	157.0 ± 22.1	298.0 ± 38.6
		%, mean	0.59	1.12
Gel IV	DHA	Mass ($\mu g\,cm^{-2}$)	1.11 ± 0.09	3.63 ± 0.07
(fish oil, cineole, ketoprofen)	(2.84 mg/8.66 μmol)	Moles ($nmol\,cm^{-2}$)	3.40 ± 0.03	11.1 ± 0.2
		%, mean	0.04	0.13
	EPA	Mass ($\mu g\,cm^{-2}$)	5.52 ± 0.11	7.03 ± 0.11
	(4.46 mg/14.8 μmol)	Moles ($nmol\,cm^{-2}$)	18.30 ± 0.36	23.4 ± 0.4
		%, mean	0.12	0.16
	Ketoprofen	Mass ($\mu g\,cm^{-2}$)	33.3 ± 4.8	57.8 ± 7.8
	(6.75 mg/26.6 μmol)	Moles ($nmol\,cm^{-2}$)	131.0 ± 18.9	228 ± 31
		%, mean	0.49	0.86
Oruvail	Ketoprofen	Mass ($\mu g\,cm^{-2}$)	67.6 ± 11.0	111.0 ± 15.1
(ketoprofen)	(3.38 mg/13.3 μmol)	Moles ($nmol\,cm^{-2}$)	266.0 ± 43.3	437 ± 59
		%, mean	2.0	3.3

Data are means $\pm$ s.d., n = 6.

Table 3 Comparison of ratios of moles permeated at 12 and 24 h

Gel	DHA/EPA[a]		(DHA + EPA)/KETO[b]	
	12 h	24 h	12 h	24 h
II (fish oil, cineole)	0.15	0.27	—	—
III (fish oil, ketoprofen)	0.20	0.40	0.18	0.16
IV (fish oil, ketoprofen, cineole)	0.19	0.47	0.17	0.15

[a]DHA to EPA for gels II, III and IV; [b]total DHA + EPA to ketoprofen (KETO) for gels III and IV.

inflammatory mediator (Ziboh et al 2000). The topical application of fish oil to viable skin was also found to generate this metabolite, although at a low proportion of $\sim$0.1% of the applied dose (Thomas & Heard, unpublished data); the same data also indicated that the use of a cetrimide receptor phase inhibited the production of 15-HEPE.

Ketoprofen was found to permeate skin from the PLO-based gels but again at low amounts. Kruskal–Wallis testing provided P values of 0.0004 and 0.0011 at 12 and 24 h, respectively. The maximum permeation was with the gel lacking 1,8-cineole ($76\,\mu g\,cm^{-2}$ (1.12%) @ 24 h vs $58\,\mu g\,cm^{-2}$ (0.86%) with 1,8-cineole) ($P > 0.05$). A large difference was observed with Oruvail where, at 24 h, $111\,\mu g\,cm^{-2}$, were delivered – this equates to 3.3% of the applied dose as the amount of ketoprofen was half that used in the PLO gels where $P \leq 0.001$ (also $P < 0.001$ @12 h). Table 3 shows that between 12 and 24 h the ratio of total DHA and EPA to ketoprofen was unchanged whether 1,8-cineole was present or absent, indicating the existence of long-chain fatty acid/ketoprofen complexes (Heard et al 2003) and that these were unaffected by the presence of the enhancer (Thomas & Heard 2005).

Conclusions

A PLO-based gel is capable of delivering EPA and DHA via a repeat finite dosing regimen, although there is evidence for the retention of these very lipophilic molecules within the gel matrix. This is a double-edged sword in that it may be beneficial for dermatological use (currently under examination) but probably would not provide sufficient delivery for use in arthritis. Although to a lesser extent than EPA and DHA, ketoprofen was also substantially retained, as exemplified by the superior delivery rate from Oruvail. The fact that EPA and DHA were found to permeate skin from massaged

multiple finite doses, but not from infinite doses, highlights the importance of using the appropriate in-use drug delivery model.

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