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In vitro percutaneous absorption studies and in vivo evaluation of anti-inflammatory activity of essential fatty acids (EFA) from fish oil extracts

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Abstract

The aim of the present study was to evaluate the in vitro percutaneous absorption and the in vivo anti-inflammatory activity of EPA and DHA fatty acids from three oily extracts, obtained by acetonic extractions from the entrails of different varieties of Mediterranean fishes such as mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*) and horse mackerel (*Trachurus mediterraneus*).

In the first part of our research, we focused our attention on the characterization of the oily extracts to determine their ω-3 polyunsaturated fatty acid content, then, we evaluated the in vitro percutaneous absorption through excised human skin (stratum corneum/epidermis membranes; SCE) of EPA and DHA contained in the extracts. In the second part, the fish oil which guaranteed the best in vitro permeation profile of these ω-3 fatty acids was studied in order to evaluate its inhibiting ability towards the in vivo UVB-induced skin erythema.

From the results obtained, all the fish oils tested in this study presented significant amounts of ω-3 fatty acids EPA and DHA, and particularly sardine oil extract showed higher concentrations of these substances compared to the other two fish oils. The in vitro experiments revealed interesting fluxes of these compounds from sardine extract through the stratum corneum/epidermis membranes and an appreciable anti-inflammatory activity against UVB-induced erythema in human volunteers was also observed.

Keywords: EPA; DHA; Anti-inflammatory activity; In vitro and in vivo studies; Skin

1. Introduction

Fish oils are a main source of ω-3 fatty acids whereas the major polyunsaturated fatty acids components (PUFA) are eicosapentaenoic acid (EPA) and

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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.

The ω-3 PUFA are mainly known for their anti-inflammatory effects related to their competition as substrates for cyclooxygenase (COX) and lipoxygenase (LOX) leading to the formation of less active prostaglandins and leukotrienes (James et al., 2000; Ziboh et al., 2000).

More recent studies assessed that ω-3 polyunsaturated fatty acid supplementation could be helpful against many inflammatory diseases (Barbosa et al., 2003; Roynette et al., 2004; Remans et al., 2004; Curtis et al., 2000). It is especially reported that dietary supplementation of fish oil, characterized by an high content of ω-3 polyunsaturated fatty acids, reduced UVB erythemal sensitivity (Rhodes et al., 1994), probably by decreasing UVB-induced prostaglandin E_2 (PGE_2) levels into the skin. However, dietary supplementation with fish oil is not always effective whereas local application of these products may be favourable to the treatment of localized inflammatory diseases as arthritis (Heard et al., 2003). Besides, some scientific works (Thomas and Heard, 2005; Heard et al., 2003; Loftsson et al., 1995) outlined a high epithelial penetration ability of PUFA and an interesting enhancement effect in the skin drug permeation.

Heard and co-workers, for instance, demonstrated in vitro the feasibility of the simultaneous permeation of ketoprofen, ibuprofen and essential fatty acids, EPA and DHA from a formulation containing fish oil. The authors in particular outlined an appreciable permeation rate of EPA and DHA through excised full-thickness pig ear skin membranes.

In the present study, we employed three oily extracts, obtained by acetonic extractions from the entrails of different varieties of Mediterranean fishes such as mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*) and horse mackerel (*Trachurus mediterraneus*).

The fatty acids composition of fish lipids, especially those of PUFAs, is species-specific and is correlated to various factors, including dietary, geographic and environmental factors, reproductive season, fishery period, etc. (Winston and Di Giulio, 1991).

The varieties of fishes chosen in this study are the most common in the market for human consumption and present a very high PUFA content (Passi et al., 2002) probably because of the geographic position of the Mediterranean basin.

In the first part of our research, we focused our attention on the characterization of the oily extracts to determine their ω-3 polyunsaturated fatty acid content and to also obtain information on their chemical composition.

Then, we evaluated the in vitro percutaneous absorption through excised human skin of EPA and DHA contained in the extracts and the fish oil that guaranteed the best in vitro permeation profile of these ω-3 fatty acids was studied in order to evaluate its inhibiting ability towards the in vivo UVB-induced skin erythema. Furthermore, the anti-erythematous efficacy of the fish oil was compared to the one observed with formulations containing ketoprofen (2%, w/w), a well-known NSAID, widely employed in the treatment of skin inflammatory diseases.

2. Materials and methods

2.1. Materials

Mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*) and horse mackerel (*Trachurus mediterraneus*) were purchased from a local store. PPG-15 stearyl ether, isohexadecane/PPG-15 stearyl ether, steareth 2, steareth 21, stearic acid, cetylstearylic acid, xanthan gum and undebenzophenon were purchased from Gattefossè (Gattefosse' Italia S.r.l., Milan, Italy). Ketoprofen was purchased by Sigma–Aldrich (Sigma–Aldrich S.r.l., Milan, Italy). All the other reagents were of analytical grade.

2.2. Extraction of fish oils

Fishes were cleaned, scaled and dissected. Heads, tissue, tails and fins were removed and discarded. Only the entrails were submitted to the extraction. The extraction of fish entrails was carried out in 2 L controlled temperature reactor and acetone was the solvent employed. Briefly, 100 g of starting fresh material was treated with 700 ml of acetone. Extraction was conducted under inert atmosphere (N_2) and at temperature of 5 °C overnight and under continuous agitation. The mixture obtained was filtered through sintered glass

filter (100–160 μm pore size) to remove the solid material. After the separation, the residue was washed with pure acetone to obtain more lipids. The combined filtrates were evaporated under reduced pressure at a temperature of 30 °C. The water residue after evaporation was separated from the oily phase by centrifugation at low temperature. Then the oil was warmed up at about 100 °C for around 15 min under inert atmosphere to get off organic solvent traces. Finally, three oily extracts were obtained: MACK, SARD and TRAC, respectively, from the entrails of mackerel, sardine and horse mackerel.

The yield of extraction process was 10.6% (w/w) for MACK, 5.7% (w/w) for SARD and 6.5% (w/w) for TRAC extracts.

2.3. Characterization of fish oils

Fatty acid composition of MACK, SARD and TRAC extracts was determined after methyl esterification according to methods of AOAC (Firestone, 1995) on a TRACE GC gas chromatograph (Thermofinnigan Inc., USA) equipped with an AS2000 auto injector (Thermofinnigan Inc., USA), a flame ionization detector and a capillary column injection system (split mode preferred at split ratio of 1:50). The separation was done on FAMEWAX capillary column (Crossbond-PEG, 30 m × 0.25 mm i.d., Superchrom S.r.l., Milan). The oven program was 170 °C (1 min), 170–225 (1 °C/min). The carrier gas helium was at flow 1.2 ml/min. and the injector and detector were held at 270 °C. The identification were done by comparison with the retention time of methyl ester standards (Supelco PUFA marine oil). The method used for the analyses determined the area percentages of fatty acids and 23:0 methyl ester was employed as internal standards for quantification of absolute weights (mg/g sample) of DHA and EPA. All values represent the mean of three analytical replications.

2.4. In vitro studies

2.4.1. Skin membrane preparation

Samples of adult human skin (mean age 36 ± 8 years) were obtained from breast reduction operations. Subcutaneous fat was carefully trimmed and the skin was immersed in distilled water at 60 ± 1 °C for 2 min (Kligman and Christophers, 1963), after which stratum corneum and epidermis (SCE) were removed from the dermis using a dull scalpel blade. Epidermal membranes were dried in a desiccator at approx. 25% relative humidity (RH). The dried samples were wrapped in aluminum foil and stored at 4 ± 1 °C until use. Preliminary experiments were carried out in order to assess SCE samples for barrier integrity by measuring the in vitro permeability of [^{3}H] water through the membranes using the Franz cells described below. The value of the permeability coefficient (P_m) for tritiated water was found to be $1.6 \pm 0.2 \times 10^{-3}$ cm/h which agreed well with those for tritiated water reported by others using human SCE samples (Bronaugh et al., 1986).

2.4.2. Skin permeation experiments

Samples of dried SCE were rehydrated by immersion in distilled water at room temperature for 1 h before being mounted in Franz-type diffusion cells supplied by LGA (Berkeley, CA). The exposed skin surface area was 0.75 cm^2 and the receiver compartment volume was of 4.5 ml.

The receptor compartment contained a water–ethanol solution (50:50), to allow the establishment of the "sink condition" and to sustain permeant solubilization (Touitou and Fabin, 1988), was stirred and thermostated at 35 ± 1 °C during all the experiments. Butylated hydroxyl anisol (0.05% w/v) was added to the donor and receptor phases to inhibit oxidation of the polyunsaturated fatty acids.

Approximately, 200 mg of each oily extract (MACK, SARD and TRAC) was placed on the skin surface in the donor compartment and the latter was covered with Parafilm. Each experiment was run in duplicate for 24 h using three different donors ($n = 3$). At intervals, whole receptor phases were removed and replaced with fresh solution. The samples were analyzed for EPA and DHA content by GC as described before. EPA and DHA fluxes through the skin were calculated by plotting the cumulative amount of active compound penetrating the skin against time and determining the slope of the linear portion of the curve and the χ-intercept values (lag time) by linear regression analysis. The fluxes (μg/(cm^2 h)), at steady state, were calculated by dividing the slope of the linear portion of the curve by the area of the skin surface through which diffusion took place.

Table 1
Composition of the topical formulations containing ketoprofen 2% (KETO) or without active compound (CONTROL)

Formulation	Oil phase	Aqueous phase	Surfactants and structurizing agents
KETO	PPG-15 stearyl ether (7 g); isohexadecane/PPG-15 stearyl ether (3 g); ketoprofen (2 g)	Distilled water (76.7 g)	Steareth 2 (3.5 g); steareth 21 (2.5 g); stearic acid (2.5 g); cetylstearylic acid (2.1 g); xanthan gum (0.3 g); undebenzophenon (0.4 g)
CONTROL	PPG-15 stearyl ether (8 g); isohexadecane/PPG-15 stearyl ether (4 g)	Distilled water (76.7 g)	Steareth 2 (3.5 g); steareth 21 (2.5 g); stearic acid (2.5 g); cetylstearylic acid (2.1 g); xanthan gum (0.3 g); undebenzophenon (0.4 g)

2.5. In vivo evaluation of photoprotective effect of fish oil extract

2.5.1. Preparation of the formulations

A ketoprofen-loaded topical formulation (KETO) was prepared to compare its anti-inflammatory efficacy to the activity of SARD fish oil. The composition of KETO formulation is reported in Table 1. The O/W emulsion was prepared by slowly adding the aqueous phase to the oily phase and to the blend of surfactants under continuous agitation: the phases were kept to 70 °C. This mixture was stirred until it was cool, thus forming the emulsion formulation. Furthermore a formulation containing the NSAID (2%, w/w) and SARD fish oil (KETOSARD) was prepared in order to evaluate the vehicle effect on ketoprofen anti-inflammatory profile.

2.5.2. Instrument

UVB-induced skin erythema was monitored, as previously reported (Bonina et al., 2001, 2002), by using a reflectance visible spectrophotometer X-Rite mod. 968 (X-Rite Inc., Grandville, MI) having 0° illumination and 45° viewing angle. The instrument was calibrated with a supplied white standard traceable to the National Bureau of Standard's perfect white diffuser. The spectrophotometer was controlled by a computer, which performed all color calculations from the spectral data by means of a menu-driven suite of programs (Spectrostart, X-Rite Inc.) supplied with the instrument. Reflectance spectra were obtained over the wavelength range of 400–700 nm using illuminant C and 2° standard observer.

2.5.3. Protocol

Experiments were performed on ten volunteers of both sexes in the age range of 25–35 years. They were recruited after medical screening including filling in a health questionnaire followed by physical examination of the application sites. After they were fully informed of the nature of the study and of the procedures involved they gave their written consent. The participants did not suffer from any ailment and were not on any medication at the time of the study. They were rested for 15 min prior to the experiments and room conditions were set at 22 ± 2 °C and 40–50% relative humidity.

Skin erythema was induced by UVB irradiation using an ultraviolet lamp mod. UVM-57 (UVP, San Gabriel, CA). For each subject, the minimal erythemal dose (MED) was preliminarily determined and an irradiation dose corresponding to the double of the MED was used throughout the study.

For each subject, eight sites on the ventral surface of the forearms were defined using a circular template (1 cm^2) and demarcated with permanent ink. Skin sites were exposed to UVB irradiation and then a weighted amount (200 mg) of SARD extract, KETO and KETOSARD formulations were spread uniformly on six sites (each formulation in twice) by means of a solid glass rod. For each volunteer, two of the eight sites were used as control and treated with a formulation without active compounds whose composition is reported in Table 1. Each skin site, after the application, was occluded for three hours, using Hill Top chambers (Hill Top Research Inc., Cincinnati, OH). After the occlusion period, the chambers were removed and the induced erythema was monitored for 58 h by reflectance spectrophotometry. From the reflectance spectral data obtained, the erythema index (EI) was calculated using an equation similar to that reported by others (Dawson et al., 1980):

$$\mathrm{EI} = 100\left[\log\frac{1}{R_{560}} + 1.5\left(\log\frac{1}{R_{540}} + \log\frac{1}{R_{580}}\right) - 2\left(\log\frac{1}{R_{510}} + \log\frac{1}{R_{610}}\right)\right]$$

where 1/*R* is the inverse reflectance at a specific wavelength (560, 540, 580, 510 and 610 nm).

EI baseline values were taken at each designed sites for formulations before UVB irradiation and they were subtracted from the EI values obtained after UVB exposure at each time point, to obtain ΔEI values. For each site, plotting ΔEI versus time the area under the curve ($AUC_{0–58}$) was computed using the trapezoidal rule.

2.6. Statistical analysis

Statistical analysis of in vitro data was performed using Student's *t*-test. Statistical differences of in vivo data are determined using repeated measure analysis of variance (ANOVA) followed by the Bonferroni–Dunn post hoc pair-wise comparison procedure. A probability, *p*, of less than 0.05 is considered significant in this study.

3. Results

3.1. Characterization of fish oils

Table 2 reports the fatty acid composition of SARD, TRAC and MACK oils (expressed as % areas). Looking at the GC analyses it was found that all the extracts showed appreciable amounts of EPA and DHA fatty acids, but the SARD extract showed the highest amount of ω-3 fatty acids. In particular from the GC analyses the DHA concentration in all the extracts was higher than the amount of EPA and also the ω-3 fatty acid contents were higher than the total ω-6 amount, confirming the information reported in literature (Passi et al., 2002). Furthermore a good amount of palmitic (C16:0) and oleic (C18:1 *n*-9) acids in all the extracts was also found.

Table 2
Fatty acid composition of SARD, TRAC and MACK oils (% areas)

Fatty acids	SARD	TRAC	MACK
C14:0	3.4 ± 0.28	2.9 ± 0.09	4.9 ± 0.08
C16:0	14.0 ± 0.11	16.3 ± 0.13	22.7 ± 0.08
C16:1	2.6 ± 0.16	5.7 ± 0.04	3.5 ± 0.11
C18:0	0.7 ± 0.01	0.8 ± 0.02	1.6 ± 0.35
C18:1 *n*9	10.9 ± 0.04	17.9 ± 0.06	15.9 ± 0.07
C18:1 *n*7	3.3 ± 1.02	3.6 ± 0.02	3.3 ± 0.01
C18:2 *n*6	1.5 ± 0.13	1.1 ± 0.01	1.7 ± 0.01
C18:4 *n*3	2.4 ± 0.08	1.0 ± 0.01	0.9 ± 0.03
C20:1 *n*9	0.8 ± 0.10	0.7 ± 0.04	1.6 ± 0.01
C20:5 *n*3 (EPA)	11.2 ± 0.66	7.2 ± 0.04	6.8 ± 0.08
C22:5 *n*3	1.4 ± 0.16	2.5 ± 0.03	1.6 ± 0.14
C22:6 *n*3 (DHA)	23.6 ± 0.2	18.4 ± 0.08	16.5 ± 0.17
Total PUFA	40.4	30.3	27.5
Total *n*3	38.6	29.1	25.8
DHA/EPA	1.99	1.4	0.9

3.2. In vitro skin permeation experiments

In vitro skin permeation experiments were performed by using SCE membranes instead of full-thickness skin since as reported by others (van de Sandt et al., 2004), the dermis in vitro can act as a significant artificial barrier to the absorption of lipophilic compounds.

The flux values at the steady state of EPA and DHA from SARD, TRAC and MACK oily extracts, calculated from the linear segments at the steady state, are reported in Fig. 1.

Statistical analysis revealed no significant differences between the steady state flux value obtained with TRAC extract and the value registered with MACK oil extract ($p > 0.05$). Moreover both extracts showed flux values considerably lower than SARD fish oil ($p < 0.01$).

3.3. In vivo anti-inflammatory activity

Since the results obtained in vitro were very promising, we thought worthwhile investigating the topical anti-inflammatory activity of SARD fish oil showing the best in vitro results. Furthermore this activity was compared to that of topical formulations containing the model drug ketoprofen widely used in the treatment of topical inflammatory diseases. For this reason,

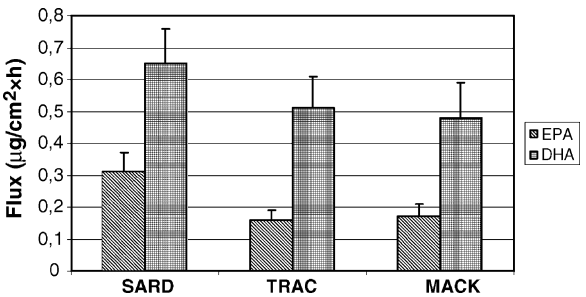

Fig. 1. EPA and DHA steady state fluxes through excised human skin from SARD, TRAC and MACK fish oils.

the 2% of ketoprofen was included in a O/W emulsion (KETO) and in SARD fish oil (KETOSARD), in order to evaluate the vehicle effect and the influence of the fish oil on ketoprofen anti-inflammatory profile.

Skin reflectance spectrophotometry enabled us to monitor in a non-invasive manner, during the in vivo experiment, the extent of the UVB-induced erythema.

Reflectance filter colorimetry has been extensively used (Westerhof et al., 1986) to designate the extent of erythema by measuring the skin color surface in term of Commission International d'Eclairage (CIE) $L^*a^*b^*$ color space parameters, since some authors (Muizzuidin et al., 1990; Braue et al., 1990) found a significant correlation between a^* values and visual grading of skin erythema. Reflectance spectrometry provided skin reflectance spectra, generally in the range 400–700 nm, which allowed us to obtain erythema index values for more accurate and reliable evaluations of skin erythema (Anderson and Parrish, 1981). From the ΔEI values, calculated at each site and at different times, it was possible to monitor the extent of UVB-induced skin erythema and the ability of the formulation tested to inhibit this process.

The ΔEI values were plotted versus time, obtaining a curve which was computed using the trapezoidal rule. The AUC values obtained are reported in Table 3.

As can be seen, all the formulations were able to inhibit UVB-induced skin erythema, since the AUC values obtained from treated skin sites were lower and significantly different from those registered with the sites treated with CONTROL formulation ($p < 0.05$). Furthermore the AUC values obtained for the skin sites treated with SARD, KETO and KETOSARD were significantly different ($p < 0.05$).

Table 3
$AUC_{0–58}$ values obtained by applying SARD fish extract and KETO and KETOSARD formulations to UVB exposed skin sites

Subjects	CONTROL	KETO	SARD	KETOSARD
A	1078.2	612.1	728.6	436.1
B	980.4	578.6	791.1	385.8
C	1170.3	583.2	866.3	421.3
D	1136.2	615.3	824.5	447.2
E	828.6	548.8	817.3	381.0
F	1030.2	497.3	802.4	455.2
G	1053.9	591.2	805.0	471.3
H	1274.6	517.3	749.6	394.6
I	1110.1	506.4	718.3	380.0
L	927.4	602.3	917.1	401.1
Mean	1059.0	565.3	802.0	417.4

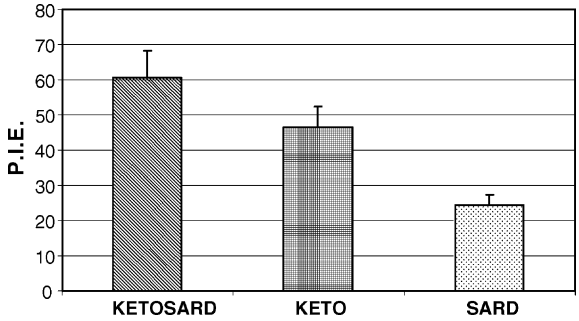

Fig. 2. Percentage inhibition of erythema (PIE) values.

In order to better outline the results obtained, from [AUC] values it is possible to calculate the percentage of inhibition of the erythema (PIE) induced by UVB radiations using the following equation:

$$\text{Inhibition}\,(\%) = \frac{\text{AUC}_\text{C} - \text{AUC}_\text{T}}{\text{AUC}_\text{C}} \times 100$$

where AUC_C is the area under the response–time curve on the vehicle treated site (control), AUC_T is the area under the response–time curve on the formulation treated site.

Fig. 2 reports the PIE values calculated for SARD oily extract and KETO and KETOSARD formulation. As demonstrated by these findings, KETOSARD appears to possess a greater inhibitory effect against skin UVB-induced erythema (PIE = 60.5%) than that elicited by SARD (PIE = 24.5%) and KETO (PIE = 46.6%).

4. Discussion

The first step of our research focused on the characterization of three oily extracts obtained from the entrails of well-known fishes of Mediterranean basin. The choice to employ the fish entrails in our study was not accidental as well as the choice to select these varieties of blue fishes. From an unpublished work previously done by us we performed, in fact, a screening among different varieties of Mediterranean fishes and we concluded that mackerels, sardines and thracuruses showed a significant amount of PUFA. Besides, our work, pointed out remarkable differences in PUFA con-

tent among tissues and entrails; the latter, particularly, after acetonic extraction, guaranteed high levels of fish oil enriched with EPA and DHA fatty acids.

The characterization performed on the three oily extracts revealed that the total amount of PUFA contained in SARD extract was higher than the one obtained from the other two fish oil varieties. Furthermore ω-6 fatty acids content was found to be lower than the total ω-3 amount (see Table 2). The data is very important since ω-6 fatty acids are endowed with a proinflammatory activity (Bagga et al., 2003; James et al., 2000), which could have influenced our in vivo experiments regarding the evaluation of the anti-inflammatory activity of the fish extract.

As for the in vitro study, in a recent work, it was demonstrated (Heard et al., 2003) that EPA and DHA permeated the skin when they are dosed in a fish oil, though the authors performed the in vitro study employing different experimental conditions compared to those used in our study. Our in vitro results showed that the permeation rate of EPA and DHA was higher from SARD oily extract compared to the other two fish extracts. This evidence could be justified by the different composition of the three extracts and probably by the total amount of PUFA contained in them. SARD extract, which guaranteed the best permeation profile of EPA and DHA, was studied in order to determine its in vivo anti-inflammatory activity after topical application on skin sites in which an erythema was previously induced by means of a UVB lamp. Furthermore, we thought it was worthwhile to compare this activity with the one elicited by two different formulations containing ketoprofen, a NSAID widely employed in the treatment of topical inflammatory diseases.

In vivo results showed that SARD was able to inhibit the UVB-induced erythema though it appeared less effective than the two formulations (KETO and KETOSARD) containing the 2% of ketoprofen and particularly KETOSARD formulation, characterized by 2% of ketoprofen in SARD extract, proved to possess the highest inhibitory power. In our opinion, this result could be justified by the anti-inflammatory activity of the two components (ketoprofen and SARD extract) and the enhancer activity of EPA and DHA contained in the formulation. So these two ω-3 fatty acids besides having an intrinsic anti-inflammatory activity they also influence the permeation rate of ketoprofen from KETOSARD formulation. The potential of EPA and DHA to act as skin penetration enhancers has been studied by other researchers (Heard et al., 2003) who assessed that the promoting effect of these ω-3 fatty acids might depend on the interaction with the permeant. Interestingly the authors observed that this interaction could strongly influence the permeation rate of EPA and DHA too.

Our data is not enough to give a full explanation of the mechanism through which EPA and DHA act as ketoprofen permeation enhancers, even because the first aim of this work was to determine the anti-inflammatory activity of these substances. However, works are in progress in order to obtain clearer information on the mechanism involving the enhancer's activity of these ω-3 fatty acids.

In conclusion, the fish oil extracts tested in this study presented a significant amount of EPA and DHA fatty acids and particularly SARD extract showed higher concentrations of these substances compared to the other two oils. The in vitro experiments revealed interesting fluxes of these compounds from SARD extract through the SCE membranes and a remarkable anti-inflammatory activity against UVB-induced erythema in human volunteers. Therefore, the present results suggest a new opportunity for fish oils and for ω-3 fatty acids to be employed as ingredients of topical pharmaceutical and cosmeceutical products employed in the treatment of skin inflammation.

References

Anderson, R.R., Parrish, J.A., 1981. The optics of human skin. J. Invest. Dermatol. 77, 13–19.

AOAC Official method—Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters in Oils and Fats, 1995. AOAC Official Methods of Analysis. In: Fireston, D. (Ed.), pp. 20–22.

Bagga, D., Wang, L., Farias-Eisner, R., Glaspy, J.A., Reddy, S.T., 2003. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proc. Natl. Acad. Sci. U.S.A. 100, 1751–1756.

Barbosa, D.S., Cecchini, R., El Kadri, M.Z., Rodriguez, M.A., Burini, R.C., Dichi, I., 2003. Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty acids. Nutrition 19, 837–842.

Bonina, F., Puglia, C., Ventura, D., Aquino, R., Tortora, S., Sacchi, A., Saija, A., Tomaino, A., Pellegrino, M.L., de Caprariis, P., 2002. In vitro antioxidant and in vivo photoprotective effects of a lyophilised extract of *Capparis spinosa* L. buds. J. Cos. Sci. 53, 321–335.

Bonina, F.P., Puglia, C., Barbuzzi, T., de Caprariis, P., Palagiano, F., Rimoli, M.G., Saija, A., 2001. In vitro and in vivo evaluation of polyoxyethylene esters as dermal prodrugs of ketoprofen, naproxen and diclofenac. Eur. J. Pharm. Sci. 14, 123–134.

Braue Jr., E.H., Mershon, M.M., Wade, J.V., Litchfield, M.R., 1990. In vivo assessment of vescicant skin injury using Minolta Chroma Meter. J. Soc. Cos. Chem. 41, 259–265.

Bronaugh, R.L., Stewart, R.F., Simon, M., 1986. Methods for in vitro percutaneous absorption studies VII: use of excised human skin. J. Pharm. Sci. 75, 1094–1097.

Curtis, C.L., Hughes, C.E., Flannery, C.R., Little, C.B., Harwood, J.L., Caterson, B., 2000. *n*-3 Fatty acids-specifically modulate catabolic factors involved in articular cartilage degradation. J. Biol. Chem. 275, 721–724.

Dawson, J.B., Barker, D.J., Ellis, D.J., Grassam, E., Catterill, J.A., Fischer, G.W., Feather, J.W., 1980. A theoretical and experimental study of light absorption and scattering by in vivo skin. Phys. Med. Biol. 25, 696–709.

Heard, C.M., Gallagher, S.J., Harwood, J., Maguire, P.B., 2003. 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? Int. J. Pharm. 261, 165–169.

James, M.J., Gibson, R.A., Cleland, L.G., 2000. Dietary polyunsaturated fatty acids and inflammatory mediator production. Am. J. Clin. Nutr. 71, 343S–348S.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88, 702–705.

Loftsson, T., Gudmundsdottir, T.K., Fridriksdottir, H., Sigurdardottir, A.M., Thorkelsson, J., Gudmundsson, G., Hjaltason, B., 1995. Fatty acids from cod-liver oil as skin penetration enhancers. Pharmazie 50, 188–190.

Muizzuidin, N., Marenus, K., Maes, D., Smith, W.P., 1990. Use of chromameter in assessing the efficacy of anti-irritants and tanning accelerators. J. Soc. Cos. Chem. 41, 369–378.

Passi, S., Cataudella, S., Di Marco, P., De Simone, F., Rastrelli, L., 2002. Fatty acid composition and antioxidant levels in muscle tissue of different Mediterranean marine species of fish and shellfish. J. Agric. Food Chem. 50, 7314–7322.

Remans, P.H., Sont, J.K., Wagenaar, L.W., Wouters-Wesseling, W., Zuijderduin, W.M., Jongma, A., Breedveld, F.C., Van Laar, J.M., 2004. Nutrient supplementation with polyunsaturated fatty acids and micronutrients in rheumatoid arthritis: clinical and biochemical effects. Eur. J. Clin. Nutr. 58, 839–845.

Rhodes, L.E., O'Farrell, S., Jackson, M.J., Friedmann, P.S., 1994. Dietary fish-oil supplementation in humans reduces UVB–erythemal sensitivity but increases epidermal lipid peroxidation. J. Invest. Dermatol. 103, 151–154.

Roynette, C.E., Calder, P.C., Dupertuis, Y.M., Pichard, C., 2004. *n*-3 Polyunsaturated fatty acids and colon cancer prevention. Clin. Nutr. 23, 139–151.

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

Touitou, E., Fabin, B., 1988. Altered skin permeation of a highly lipophilic molecule: tetrahydrocannabinol. Int. J. Pharm. 43, 17–22.

van de Sandt, J.J., van Burgsteden, J.A., Cage, S., Carmichael, P.L., Dick, I., Kenyon, S., Korinth, G., Larese, F., Limasset, J.C., Maas, W.J., Montomoli, L., Nielsen, J.B., Payan, J.P., Robinson, E., Sartorelli, P., Schaller, K.H., Wilkinson, S.C., Williams, F.M., 2004. In vitro predictions of skin absorption of caffeine, testosterone and benzoic acid: a multi-centre comparison study. Regul. Toxicol. Pharmacol. 39, 271–281.

Westerhof, W., Van Hasselt, B.A.A.M., Kammeijer, A., 1986. Quantification of UV-induced erythema with a portable computer chromameter. Photodermatology 3, 310–314.

Winston, G.W., Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanism in aquatic organism. Aquat. Toxicol. 19, 137–161.

Ziboh, V.A., Miller, C.C., Cho, Y., 2000. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of anti-inflammatory and antiproliferative metabolites. Am. J. Clin. Nutr. 71, 361S–366S.