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Note

Prostaglandin E_2 and interleukin-8 production in human epidermal keratinocytes exposed to marine lipid-based liposomes

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Abstract

In response to exogenous stimuli such as non-sensitizing contact irritants, human keratinocytes produce various types of soluble pro-inflammatory mediators including prostaglandin E_2 (PGE_2) and interleukin 8 (IL-8). Polyunsaturated fatty acids of the $n-3$ series ($n-3$ PUFA) are known to play a role in the prevention of the inflammatory response. In this work, $n-3$ PUFA were supplied to keratinocytes through Marinosomes® that are liposomes based on a natural marine lipid extract. Marinosomes® contributed to reduce inflammation induced by croton oil by regulating PGE_2 and IL-8 production in human keratinocyte cultures. However, the preventing effect of Marinosomes® was highly dependent on the lipid concentration used and the liposome mean diameter.

Keywords: Liposome; Polyunsaturated fatty acid; Skin metabolism

1. Introduction

The skin is the main tissue protecting us from harmful environmental hazards, UV-irradiation and endogenous water loss. In response to physiological and chemical stresses, keratinocytes produce and release various signaling factors such as inflammatory cytokines and growth factors (Coquette et al., 2003; Welss et al., 2004), as well as metabolites formed in the lipoxygenase and cyclooxygenase (COX) pathways (Fogh and Kragballe, 2000; Lee et al., 2003). Among these metabolites, prostanoids synthesized from eicosapentaenoic acid (EPA, 20:5 $n-3$) are less pro-inflammatory than those synthesized from arachidonic acid (AA, 20:4 $n-6$) (Ziboh, 1996; Ziboh et al., 2000). Thus, among the active compounds able to regulate the inflammatory response, a large attention was paid to $n-3$ polyunsaturated fatty acids (PUFA). Because the skin does not present *in vivo* $\Delta 6$ and $\Delta 5$-desaturase activities (Chapkin et al., 1986), EPA, a long chain PUFA, cannot be synthesized from its precursor α-linolenic acid (18:3 $n-3$). Thus, this fatty acid must be provided by dietary supplementation or by the topical route.

Marinosomes® have proved to be good candidates for $n-3$ PUFA topical delivery. Indeed, they exhibited a rather good stability in conditions that mimic those of the skin in terms of pH, temperature and calcium concentration (Moussaoui et al., 2002) suggesting that they could be used in view of the prevention and treatment of skin diseases. The main objective of the present study was to investigate the influence of Marinosomes®, i.e., liposomes prepared with a natural marine lipid extract rich in $n-3$ PUFA on the production of two inflammatory metabolites, prostaglandin E_2 (PGE_2), a major COX product, and interleukin 8 (IL-8), a chemotactic cytokine, generated by keratinocyte cells.

2. Materials and methods

The natural lipid mixture Phosphomins® (Phosphotech, France) used for liposome preparation contained 75 wt% phospholipids mainly represented by phosphatidylcholine (71 wt%) and phosphatidylethanolamine (18 wt%) (Nacka et al., 2001a). PUFA accounted for 56% of total phospholipid fatty acids among which EPA represented 30%. α-Tocopherol (Sigma) (5 wt%) was added to the marine lipid extract to prevent PUFA oxidation (Nacka et al., 2001b).

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Marinosomes® were prepared in 10 mM HEPES buffer (pH 7.4) after extrusion of a lipid suspension (20 g L^{-1}) through polycarbonate filters (5, 1.2 and 0.4-μm pore size, Millipore) (Nacka et al., 2001a). Liposome structural characterization was carried out by optical microscopy and freeze-fracture transmission electron microscopy (Moussaoui et al., 2002). The liposome mean diameter was determined by granulometry measurements (Mastersizer 2000, Malvern).

Normal human epidermal keratinocytes were obtained from newborn foreskin following surgery. Cells were established using the explant method and were grown in serum-free Keratinocyte Growth Medium (Clonetics®). A cell viability test was carried out by seeding transwell culture plates with 2×10^4 keratinocytes per well. The plates were incubated at 37 °C in a humidified 5% CO_2 incubator. The cells were exposed for 72 h to liposome suspensions with lipid concentrations ranging from 0.05 to 10 g L^{-1} (triplicate wells per concentration). Cell viability was assessed using the neutral red uptake (NRU) assay (Borenfreund and Puerner, 1985).

The keratinocyte cell cultures were placed in contact with the liposome suspension for 48 h prior to inflammation induction. Inflammation was induced by addition of croton oil (2×10^{-2} g L^{-1}) in the culture medium for 24 h. The pro-inflammatory biomarkers IL-8 and PGE_2 were assayed on the subnatants of the cell cultures using immunochemical tests (Kit no. 2237, Immunotech® for IL-8; Kit no. DE0100, R&D Systems for PGE_2). The cell layers were also tested for protein content (BioRad Protein assay). Triplicate wells per Marinosomes® concentration were assayed. The anti-inflammatory activity (%) was calculated as follows:

$$\frac{([X]_{\text{croton oil}} - [X]_{\text{croton}}) - ([X]_{\text{liposomes+croton oil}} - [X]_{\text{control}})}{[X]_{\text{croton oil}} - [X]_{\text{control}}} \times 100$$

X represented the biomarkers IL-8 or PGE_2; $[X]_{\text{liposomes + croton oil}}$ was the concentration of IL-8 or PGE_2 (pg μg^{-1} protein) for the cells treated with the liposomes and croton oil; $[X]_{\text{croton oil}}$ was the concentration of IL-8 or PGE_2 (pg μg^{-1} protein) for the cells treated only with croton oil. $[X]_{\text{control}}$ represented the basal concentration of IL-8 or PGE_2 (pg μg^{-1} protein) of the cells.

Analysis of variance (ANOVA), using Fisher's least significant difference (LSD) procedures (Stat-Graphics Plus software), was performed to discriminate among the liposome size and lipid concentration effects.

3. Results and discussion

Keratinocytes represent the major cell type in the epidermis and play key roles in skin inflammatory and immunological reactions. Because of their ability to produce a large range of inflammatory mediators, immersed keratinocyte cultures were chosen for the development of *in vitro* methods to test irritants. However, it is worth noting that due to the absence of the stratum corneum, the concentrations inducing responses in these monolayer cell cultures are usually several orders of magnitude lower than those which induce inflammation *in vivo* (Coquette et al., 2003).

Marinosomes® of different sizes were prepared by a simple preparation technique, i.e. extrusion. The 5-μm liposome suspension consisted of a mixture of more or less spherical vesicles composed of one or more bilayers as observed by optical microscopy and freeze-fracture transmission electron microscopy (Moussaoui et al., 2002). The liposome mean diameter was 5.3 ± 1.1 μm. The 1.2 and 0.4-μm liposome suspensions were also observed by the different microscopy techniques confirming that further extrusion decreased the vesicle size around the pore size used.

The anti-inflammatory activity of Marinosomes® of different sizes and at different concentrations was evaluated by the release of intracellular IL-8 and PGE_2 originating from by keratinocyte cells, responding to croton oil. Croton oil was used as a non-sensitizing contact irritant. The selected liposome concentrations were obtained from viability measurements: no cytotoxic effect was measured for concentrations up to 1 g L^{-1} irrespective of the liposome size (5, 1.2 or 0.4 μm). PGE_2 production was first investigated. The tetradecanoyl phorbol acetate present in croton oil activated the formation of AA metabolites and thus, accounted for PGE_2 production by keratinocytes (Liu and Belury, 1998). As expected, after the stimulation of the keratinocytes by croton oil, the baseline PGE_2 level (47.24 ± 5.34 pg μg^{-1} protein) was increased by a factor 14 (645.81 ± 15.30 pg μg^{-1} protein) (Fig. 1). Five micrometer marine lipid-based liposomes antagonized the stimulatory effect of croton oil irrespective of the concentration used. However, the anti-inflammatory activity was at its maximum for the two lowest concentrations used while it was only 51% for the highest concentration (Table 1). PGE_2 release was significantly dependent on the liposome size (Table 1). For the lowest lipid concentrations, 0.4-μm Marinosomes® were less efficient in reducing the inflammatory activity of croton oil towards PGE_2 release. For the concentration of 1 g L^{-1}, the anti-inflammatory activity was equal to about 50%, irrespective of the liposome size. It is worth noting that there was a significant interaction between the liposome size and the lipid concentration factors. Croton oil was also a strong inductor of IL-8 release (Fig. 2). The highest inhibition of IL-8 release was observed for the concentration of 1 g L^{-1} of

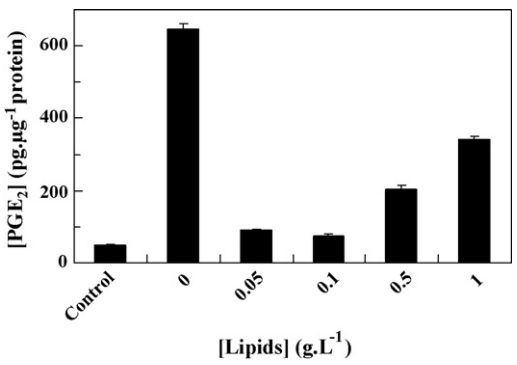

Fig. 1. PGE_2 release (pg μg^{-1} protein) measured in human keratinocytes in the basal state (control) and after their activation by croton oil. PGE_2 production by keratinocytes is expressed as means ± S.D. of triplicate wells. Marine lipid-based liposomes were added 48 h prior to croton oil addition at different lipid concentrations. Liposomes were prepared by filtration of a 20 g L^{-1} lipid suspension through polycarbonate filters (5-μm pore size).

Table 1
Influence of the liposome size on the anti-inflammatory activity (%) of keratinocytes stimulated by croton oil (20 mg L^{-1}) relatively to PGE_2 production

[Lipids] (g L^{-1})	Liposome size (μm)		
	5	1.2	0.4
0.05	92.9 ± 3.3 c, e	94.8 ± 6.3 c, f	42.2 ± 2.3 c, g
0.1	95.6 ± 7.1 c, e	86.0 ± 2.0 c, f	49.2 ± 2.6 c, g
0.5	73.9 ± 4.0 b, e	48.4 ± 2.7 b, f	59.8 ± 1.9 b, g
1	51.3 ± 1.7 a, e	44.4 ± 2.0 a, f	56.3 ± 2.9 a, g

a, b, c: different letters indicated significant lipid concentration difference ($P < 0.05$). e, f, g: different letters indicated significant liposome size difference ($P < 0.05$).

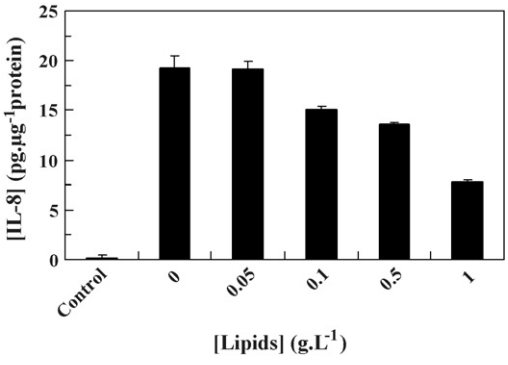

Fig. 2. IL-8 release (pg μg^{-1} protein) measured in human keratinocytes in the basal state (control) and after their activation by croton oil. IL-8 production by keratinocytes is expressed as means ± S.D. of triplicate wells. Marine lipid-based liposomes were added 48 h prior to croton oil addition at different lipid concentrations. Liposomes were prepared by filtration of a 20 g L^{-1} lipid suspension through polycarbonate filters (5-μm pore size).

marine lipid-based liposomes. The anti-inflammatory effect of Marinosomes® was somewhat dependent on the liposome mean diameter (Table 2). Except for the 0.05 g L^{-1} lipid concentration, 5-μm liposomes exhibited the best anti-inflammatory activity. No significant interaction was found between the liposome size and the lipid concentration factors.

Numerous studies showed that liposomes may interact with the different layers of the epidermis (Lasic, 1993; Bouwstra et al., 2003) although the inter- and intra-cellular modifications strongly depended on the liposome composition and involved different mechanisms. The effect of Marinosomes® on PGE_2 and IL-8 release strongly suggested that these liposomes were also able to interact with human keratinocytes. This interaction could occur either through liposome adsorption and fusion with the keratinocyte membrane cell or degradation of the liposome membrane organization and transfer of lipid species into the keratinocyte membrane. The fact that the mediator production was dependent on the liposome size may reflect an increased interaction of large Marinosomes® with keratinocytes membrane cell and/or a lower stability of small liposomes in the culture medium. The role of EPA on the modulation of inflammatory mediators induced by UVB-irradiation was shown in normal human keratinocytes (Pupe et al., 2002) and healthy skin *in vivo* (Shahbakhti et al., 2004). At low concentration of Marinosomes®, our results were consistent with the fact that EPA can be considered as a competitive inhibitor of AA conversion to PGE_2 leading to a decreased production of this prostaglandin. The fact that, at higher Marinosomes® concentration, PGE_2 secretion increased could correspond to a slight immune response of Marinosomes® as their concentration increased. It was shown that EPA pre-treatment of normal human keratinocytes resulted in an inhibition of IL-6 secretion (Pupe et al., 2002). However, in healthy skin *in vivo*, EPA did not seem to act on the reduction of sunburn through IL-8 (Shahbakhti et al., 2004). In our work, we showed that Marinosomes® were able to decrease IL-8 secretion as soon as the lipid concentration was sufficient. The fact that Marinosomes® exhibited an antagonist effect on PGE_2 and IL-8 release suggested that the factors which caused the release of these mediators did not work through the same signal mechanisms. In the case of UVB radiation, the regulation of IL-8 production seemed to be mediated via a cAMP-independent protein kinase A pathway (Grandjean-Laquerriere et al., 2005). Indeed, PGE_2 that enhances cAMP concentration, decreases IL-8 production in human keratinocyte cell line by down-regulating NF-κB transcription factor activation (Grandjean-Laquerriere et al., 2005). In conclusion, Marinosomes® could be envisaged to target the negative effects of a non-sensitizing contact irritant because they modulated PGE_2 biosynthesis and IL-8 secretion. However, parameters like lipid concentration and liposome size should to be taken into account in order to get the best human health benefit.

Table 2
Influence of the liposome size on the anti-inflammatory activity (%) of keratinocytes stimulated by croton oil (20 mg L^{-1}) relatively to IL-8 production

[Lipids] (g L^{-1})	Liposome size (μm)		
	5	1.2	0.4
0.05	0.8 ± 0.1 b, c	26.2 ± 1.1 b, c	11.0 ± 0.3 b, d
0.1	21.8 ± 0.6 b, c	15.0 ± 0.4 b, c	0.8 ± 0.1 b, d
0.5	29.7 ± 0.6 b, c	23.2 ± 0.5 b, c	4.0 ± 0.1 b, d
1	60.2 ± 2.3 a, c	34.2 ± 2.1 a, c	7.8 ± 0.2 a, d

a, b: different letters indicated significant lipid concentration difference ($P < 0.05$). c, d: different letters indicated significant liposome size difference ($P < 0.05$).

References

Borenfreund, E., Puerner, J.A., 1985. Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicol. Lett. 24, 119–124.

Bouwstra, J.A., Honeywell-Nguyen, P.L., Gooris, G.S., Ponec, M., 2003. Structure of the skin barrier and its modulation by vesicular formulations. Prog. Lipid Res. 42, 1–36.

Chapkin, R.S., Ziboh, V.A., Marcelo, C.L., Voorhees, J.J., 1986. Metabolism of essential fatty acids by human epidermal enzyme preparations: evidence of chain elongation. J. Lipid Res. 27, 945–954.

Coquette, A., Berna, N., Vandenbosch, A., Rosdy, M., De Wever, B., Poumay, Y., 2003. Analysis of interleukin-1α (IL-1α) and interleukin-8 (IL-8) expression and release in in vitro reconstructed human epidermis for the prediction of in vivo skin irritation and/or sensitization. Toxicol. In Vitro 17, 311–321.

Fogh, K., Kragballe, K., 2000. Eicosanoids in inflammatory skin diseases. Prostaglandins Other Lipid Mediators 63, 43–54.

Grandjean-Laquerriere, A., Le Naour, R., Gangloff, S.C., Guenounou, M., 2005. Contribution of protein kinase A and protein kinase C pathways in ultraviolet B-induced IL-8 expression by human keratinocytes. Cytokine 29, 197–207.

Lasic, D.D., 1993. Cosmetic applications of liposomes. In: Liposomes. From Physics to Applications. Elsevier, Amsterdam, pp. 477–488.

Lee, J.L., Mukhtar, H., Bickers, D.R., Kopelovich, L., Athar, M., 2003. Cycoloxygenases in the skin: pharmacological and toxicological implications. Toxicol. Appl. Pharmacol. 192, 294–306.

Liu, K.L., Belury, M.A., 1998. Conjugated linoleic acid reduces arachidonic acid content and PGE_2 synthesis in murine keratinocytes. Cancer Lett. 127, 15–22.

Moussaoui, N., Cansell, M., Denizot, A., 2002. Marinosomes®, marine lipid-based liposomes: physical characterization and potential application in cosmetics. Int. J. Pharm. 242, 361–365.

Nacka, F., Cansell, M., Gouygou, J.P., Gerbeaud, C., Méléard, P., Entressangles, B., 2001a. Physical and chemical stability of marine lipid-based liposomes under acid conditions. Colloids Surf. B 20, 257–266.

Nacka, F., Cansell, M., Méléard, P., Combe, N., 2001b. Incorporation of α-tocopherol in marine lipid-based liposomes. *In vitro* and *in vivo* studies. Lipids 36, 1313–1320.

Pupe, A., Moison, R., De Haes, P., van Henegouwen, G.B., Rhodes, L., Degreef, H., Garmyn, M., 2002. Eicosapentaenoic acid, a $n-3$ polyunsaturated fatty acid differentially modulates TNF-α, IL-1α, IL-6 and PGE_2 expression in UVB-irradiated human keratinocytes. J. Invest. Dermatol. 118, 692–698.

Shahbakhti, H., Watson, R.E., Azurdia, R.M., Ferreira, C.Z., Garmyn, M., Rhodes, L.E., 2004. Influence of eicosapentaenoic acid, an omega-3 fatty acid, on ultraviolet-B generation of prostaglandin-E2 and proinflammatory cytokines interleukin-1 beta, tumor necrosis factor-alpha, interleukin-6 and interleukin-8 in human skin in vivo. Photochem. Photobiol. 80, 231–235.

Welss, T., Basketter, D.A., Schröder, K.R., 2004. In vitro skin irritation: facts and future. State of the art review of mechanisms and models. Toxicol. In Vitro 8, 231–243.

Ziboh, V.A., 1996. The significance of polyunsaturated fatty acids in cutaneous biology. Lipids 31, S249–S253.

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