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Anti-inflammatory effects of eicosapentaenoic acid on experimental skin inflammation models

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Abstract. Anti-inflammatory effects of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) were examined on three models of skin inflammation induced in mice by topical application of an arachidonic acid (AA) solution, ultraviolet-B (UVB) irradiation, and contact sensitization with dinitrofluorobenzen. Ear oedema reactions induced by AA and UVB irradiation were significantly suppressed in mice fed a daily dose of 300 mg/kg EPA for 2 weeks. The contact hypersensitivity reaction was not impaired by EPA. None of the skin reactions was significantly inhibited in mice fed DHA or safflower oil. The results suggest that EPA, but not DHA, has anti-inflammatory effects on AA- and UVB-induced acute inflammation reactions.

Key words: Anti-inflammatory effect – Docosahexaenoic acid – Ear oedema – Eicosapentaenoic acid – Ultraviolet radiation

Eicosapentaenoic acid (EPA), a polyunsatulated fatty acid (20:5, n-3), is contained in large amounts in oil from fish such as sardines and mackerel. EPA is metabolized through the same enzymatic pathways that catalyse arachidonic acid (AA) (20:4, n-6) but, while AA yields biologically potent mediators such as prostaglandin (PG) E_2 and leukotriene (LT) B_4, EPA is converted to much less active derivatives such as PGE_3 and LTB_5 [18].

Dietary supplementation of EPA-rich fish oil exerts prophylactic and therapeutic effects on chronic inflammatory diseases such as arteriosclerotic diseases, rheumatoid arthritis and psoriasis [1, 9, 13, 25]. Highly purified EPA ethyl ester has recently become commercially available in Japan as a promising drug for the treatment of psoriasis [8, 22]. We have previously reported that six of 20 cases of psoriasis vulgaris markedly improved after 6 months administration of 1.8 g/day EPA [6].

As to therapeutic mechanisms, the drug has the following three major actions: (1) suppression of keratinocyte proliferation [10]; (2) anti-inflammatory effects [11, 12, 19, 21, 25]; and (3) immunosuppressive effects [7, 17, 23]. In this study, three models of skin inflammation were induced in animals for examining the anti-inflammatory effects of EPA. The effects of docosahexaenoic acid (22:6, n-3) (DHA) were also examined since DHA is an analogue of EPA.

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Materials and methods

Feeding protocol

Female BALB/c mice, weighing approximately 18 g, were fed water and regular chow (CE-2, Japan Clea) containing 4.1% crude fats consisting mainly of soybean oil. Soybean oil contains 52% linoleic acid (18:2, n-6), the dietary source of AA, and 10.9% α-linolenic acid (18:3, n-3), the precursor of EPA. The following reagents were administered orally for 2 weeks: (1) distilled water (control); (2) EPA ethyl ester freshly suspended in distilled water by sonication (30–300 mg/kg); (3) DHA ethyl ester (30–300 mg/kg); and (4) safflower oil (SO) (30–300 mg/kg). SO consists mainly of 76.4% linoleic acid and 0.2% α-linolenic acid. EPA was obtained from Mochida Pharmaceutical Company (Tokyo, Japan) and DHA and SO were kindly supplied by the Sagami Chemical Research Center (Sagamihara, Japan). On the last day of feeding, the following experiments were carried out.

Mouse ear oedema induced by arachidonic acid

Ear oedema was induced by topical application of 0.01 ml 99% acetone containing 50 mg/ml AA (grade 1, purity 99%; Sigma Chemical Company, St. Louis, Mo., USA) to both ear lobes according to our previously reported method [5]. In each animal, the increase in ear thickness (ear swelling response) was measured 1, 2 and 4 h after painting using a dial thickness gauge (Mitutoyo Corporation, Japan) and the values obtained from both ear lobes averaged. The control group comprised ten mice and each active group comprised six mice. Biopsy specimens were obtained from some mice at 1 h post-application.

Ultraviolet radiation

Mice were given a single exposure to ultraviolet-B (UVB) radiation using a bank of five fluorescent sunlamp tubes (FL-20SE; Toshiba,

Tokyo, Japan). The total exposure dose was 500 mJ/cm^2 (exposure time, 20 min). In each animal, the ear swelling response was measured 24 h after irradiation and the values obtained from both ear lobes averaged. Each group comprised six mice. Biopsy samples were taken from all mice for histological analysis.

The number of sunburn cells appearing in the epidermis was counted in haematoxylin–eosin-stained sections. Sunburn cells appear as eosinophilic ovoid bodies with or without remnants of pyknotic nuclei. In each animal, the number per millimetre of epidermis was counted and the values obtained from both ear lobes averaged.

Contact hypersensitivity

Mice were sensitized by topical application of 0.02 ml 0.5% dinitrofluorobenzene (DNFB) dissolved in a mixture of acetone and olive oil (4 : 1) to the shaved abdomen. Challenge reactions of the skin were induced 5 days after sensitization by painting 0.01 ml 0.2% DNFB solution onto both ear lobes. In each animal, the increase in ear thickness was measured the day after challenge and the values obtained from both ear lobes averaged. Each group comprised six mice. Administration of EPA and other reagents was continued until the day before challenge.

Results

Gross changes in mouse skin

Mice of both control and treated groups had gained a mean of approximately 5% in body weight at the end of the 2-week feeding period. However, mice fed EPA and DHA, but not those fed SO, lost their shiny lie of hair.

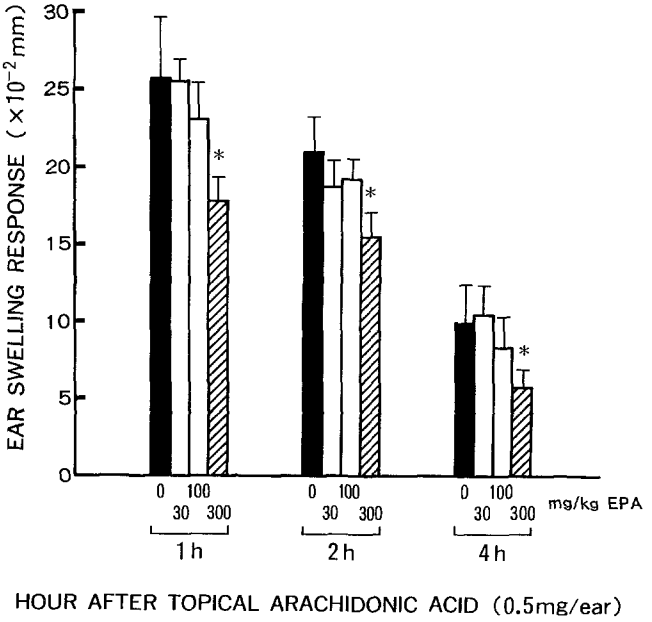

Fig. 1. Effects of eicosapentaenoic acid (EPA) on arachidonic acid-induced ear swelling response (mean ± standard deviation). *Asterisks* indicate statistically significant values ($p < 0.05$) compared with those of the control group (0 mg/kg EPA) calculated using Student's *t*-test

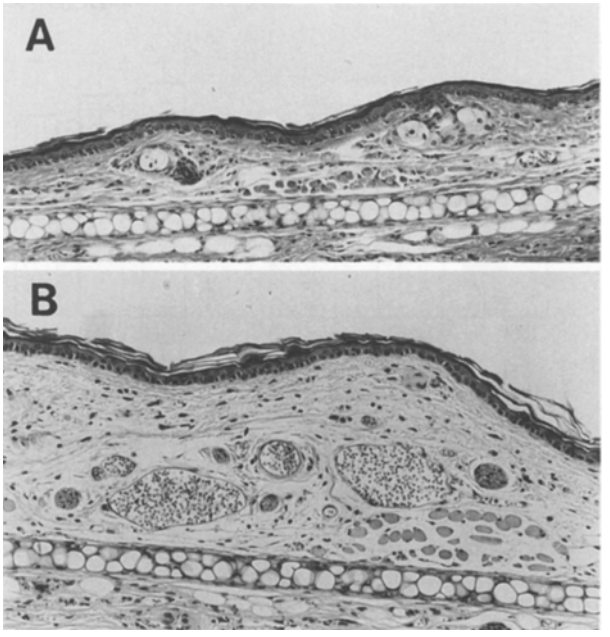

Fig. 2. Histopathological findings of a normal mouse ear lobe (**A**) and an ear lobe 1 h after painting with 0.5 mg arachidonic acid (**B**). In **B**, note marked dermal oedema and dilation of vessels packed with erythrocytes. There are few polymorphonuclear leukocytes infiltrating into the tissue. (haematoxylin-eosin; ×60)

Arachidonic acid-induced ear oedema

Ear oedema induced by AA was suppressed by EPA in a dose-dependent manner. Suppression was significant in the group of mice fed a daily dose of 300 mg/kg EPA compared with the control group (Fig. 1). No significant changes were observed in mice treated with lower doses of EPA or any dose of DHA or SO.

Histological specimens obtained from control mouse ears showed marked dermal oedema and vasodilation with few infiltrating polymorphonuclear leukocytes (Fig. 2). Ear oedema and vasodilation appeared to be less marked in the 300 mg/kg EPA-treated group, although quantitative measurements were not possible.

UVB-induced ear oedema

UVB-induced ear oedema was significantly suppressed by treatment with a daily dose of 300 mg/kg EPA, but not by 30–100 (Fig. 3). No significant changes were induced by any dose of DHA or SO.

Histologically, there were no significant changes in sunburn-cell counts between the control and any of the treated groups of mice.

Contact hypersensitivity

Contact hypersensitivity reactions induced by DNFB were not significantly suppressed by treatment with any dose of EPA. The effects of DHA and SO were not examined.

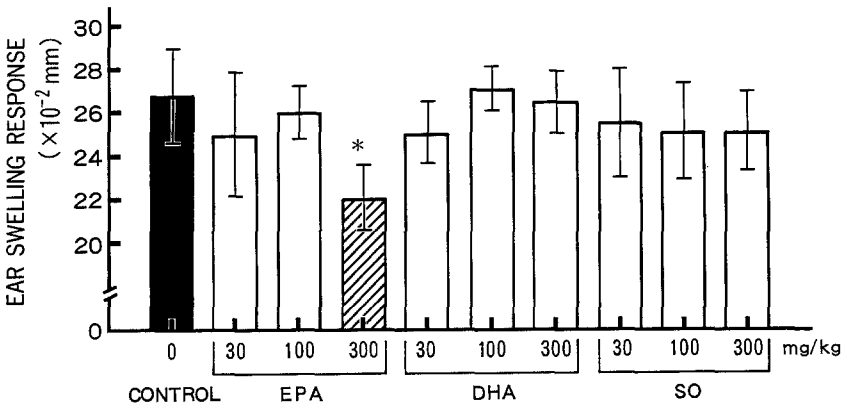

Fig. 3. Effects of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and safflower oil (SO) on and UVB-induced ear swelling response (mean ± standard deviation). The *asterisk* indicates a statistically significant value ($p < 0.01$) compared with that of the control group calculated using Student's *t*-test

Discussion

Ear oedema induced by AA is mediated by biologically active metabolites, including PGs (mainly PGE_2) and LTs (mainly LTB_4, C_4 and D_4), derived from exogenously administered AA via both cyclooxygenase and lipoxygenase pathways [15, 24]. According to biological and biochemical analyses by others, EPA competes directly with AA or EPA metabolites such as 15-hydroxyeicosapentaenoic acid and LTB_5 inhibit enzymes that catalyse the cyclooxygenase and lipoxygenase pathways [11, 12, 14, 18, 19, 25]. EPA-derived metabolites have lower biological activity than those formed through AA [20].

In our model, oral EPA increases the concentration of EPA in the skin and therefore EPA presumably interferes in the AA cascade, resulting in decreased production of the AA-derived potent inflammatory mediators involved in ear oedema reactions. Histologically, ear oedema was associated with negligible immigration of polymorphonuclear leukocytes. This finding agrees with a previous report [15] and suggests that cells responsible for the conversion of AA are mainly a resident population such as epidermal cells and mast cells, but not inflammatory cells.

The mechanism described above can also explain the suppressive effect of EPA on UVB-induced mouse ear oedema reactions because AA and its metabolites are involved in acute UVB inflammation [2, 16]. However, sunburn-cell formation, a histological parameter of UVB injury, was not affected by EPA. This is probably because sunburn cells are formed through different mechanisms including UVB-induced oxygen intermediates [4]. In the above two models, administration of SO, which is rich in AA and contains a small amount of EPA, had no measurable effects, supporting the specificity of the EPA effects.

Our findings are in accordance with an earlier observation that oral administration of EPA (240 mg/kg per day) suppresses carrageenin-induced rat foot-pad swelling reactions, in which the AA cascade is also involved [21]. The results of our and others' animal studies will, in part, underlie the clinical effectiveness of EPA in psoriasis, although the EPA doses used in the animal studies are approximately 3–10 times higher than clinical doses. EPA may compete with AA which participates in the pathogenesis of psoriasis [18].

Other effects of EPA such as anti-proliferative effects [10] and immunomodulatory effects [7, 17, 23] may possibly be related to its therapeutic mechanisms in psoriasis. In this study, the suppressive effects of EPA on contact hypersensitivity, an experimental model for cellular immunity, were tested without significant results.

The present study demonstrates that anti-inflammatory effects of DHA on skin inflammation reactions are less marked than those of EPA. This is partially because DHA has inhibitory effects on the PG synthetase pathway with negligible effects on the LT pathway [3, 11, 18]. Alternatively, the anti-inflammatory effects of DHA would be uncovered by examining them in other models.

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