

Eicosapentaenoic Acid Suppresses the Proliferation of Synoviocytes from Rheumatoid Arthritis

Masahide Hamaguchi¹, Yutaka Kawahito^{1,*}, Atsushi Omoto¹, Yasunori Tsubouchi¹, Masataka Kohno¹, Takahiro Seno¹, Masatoshi Kadoya¹, Aihiro Yamamoto¹, Hidetaka Ishino¹, Masahide Matsuyama², Rikio Yoshimura², and Toshikazu Yoshikawa¹

¹Inflammation and Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan

²Department of Urology, Osaka City University Graduate School of Medicine, Osaka 545-8585, Japan

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Summary Eicosapentaenoic acid (EPA) is essential for normal cell growth, and may play an important role in inflammatory and autoimmune disorders including rheumatoid arthritis. We investigate that EPA could suppress the proliferation of fibroblast like synoviocytes *in vitro*. We treated synoviocytes with 1 to 50 μ M EPA and measured cell viabilities by the modified MTT assay. We sorted the number of them in sub G1 stage by fluorescence-activated cell sorting caliber. And we stained them by light green or Hoechst 33258, and investigate microscopic appearance. The cell viabilities were decreased at 30 μ M, 40 μ M, and 50 μ M of EPA comparing to 0 μ M of EPA. The half maximal concentration of synoviocytes inhibition was approximately 25 μ M. At day 1 and day 3, cell number was also decreased at 50 μ M EPA comparing to control. FACS caliber indicated the number of synoviocytes in sub G1 stage did not increase in each concentration of EPA. Hoechst staining indicated normal chromatin pattern and no change in a nuclear morphology both in EPA treated synoviocytes and in untreated synoviocytes. These findings suggest that EPA could suppress the proliferation of synoviocytes *in vivo* dose dependently and time dependently, however, the mechanism is not due to apoptosis.

Key Words: eicosapentaenoic acid, rheumatoid arthritis, synoviocyte, cell growth

Introduction

Long chain omega-3 polyunsaturated fatty acids (PUFAs) including eicosapentaenoic acid (EPA) are essential for normal cell growth, and may play an important role in the prevention and treatment of coronary artery disease, hypertension, cancer, and other inflammatory and autoimmune disorders including rheumatoid arthritis [1]. The first evidence of the important role of dietary intake of PUFAs in inflammation was derived from epidemiological observa-

tions of the low incidence of autoimmune and inflammatory disorders, such as psoriasis, asthma and type-1 diabetes, as well as multiple sclerosis in a population of Inuit compared with gender- and age-matched groups living in Denmark [2]. Inuit have a high dietary intake of long chain PUFAs including eicosapentaenoic acid from seafood [3]. There are at least 13 randomized controlled clinical trials that show benefit from fish oil supplements in patients with rheumatoid arthritis (RA) [4]. A common feature of the studies has been a reduction in symptoms and in the number of tender joints. There was a reduction in the dose of analgesic anti-inflammatory drugs [5]. In a subsequent meta-analysis, morning stiffness was decreased, as well as the number of tender joints. So, we investigate that EPA could suppress the proliferation of fibroblast like synoviocytes *in vitro*.

*To whom correspondence should be addressed.

Tel: +81-75-251-5505 Fax: +81-75-252-3721

E-mail: kawahity@koto.kpu-m.ac.jp

Materials and Methods

We obtained synovial tissues from patients with RA who received replacement surgery, and used synoviocytes at passage of 3–6, when they showed fibroblastoid morphology. We measured cell viability by using modified MTT assay (WST-1 assay; Dojindo, Kumamoto, Japan). Synoviocytes were treated with 1–50 μM EPA in RPMI1640 with or without 1% heat-inactivated fetal cow serum (FCS) on 96-well plates at a density of 1×10^4 cells per plate, 35 mm diameters plates at a density of 2×10^5 cells per plate, or 8×8 mm multi chamber slides at a density of 2×10^4 cells per plate. Floating and adherent synoviocytes were harvested at 24 h later, were stained by propidium iodide and sorted with fluorescence-activated cell sorting (FACS) caliber.

Second, we investigate microscopic appearance of synoviocytes cultured with 50 μM of EPA and RPMI1640 with 1% FCS and onto 8×8 mm multichamber slides at a density of 2×10^4 cells per plate. Adherent synoviocytes were harvested at 24 h later and were stained by 0.5% light green. We stained DNA chromatin by Hoechst 33258 (Dojindo).

Statistical Analysis

The cell viabilities of synoviocytes treated with 1–50 μM EPA were analyzed by the Kruskal-Wallis test followed by Dunn's multiple comparison test. The cell viabilities and cell number of synoviocytes treated with or without 50 μM EPA were analyzed by paired *t* test at day 0, 1, 3, and 5. $p < 0.05$ was considered significantly different.

Results

The cell viabilities were decreased at 30 μM , 40 μM , and 50 μM of EPA comparing to 0 μM of EPA ($p < 0.001$, Fig. 1A and 1B). Twenty-four hours later, the number of synoviocytes was reduced the control group, and its half maximal concentration of synoviocytes inhibition was approximately 25 μM (Fig. 2). At day 1 and day 3, cell number was also decreased at 50 μM EPA comparing to control (Fig. 3). FACS caliber indicated the number of synoviocytes in sub-G1 stage didn't increase in each concentration of EPA.

At 24 h later EPA-treated synoviocytes appears less active and had an irregular or round shape compared with untreated synoviocytes. Hoechst staining indicated normal chromatin pattern and no change in a nuclear morphology both in EPA-treated synoviocytes and in untreated synoviocytes.

Discussion

Our results suggest that EPA could suppress the proliferation of synoviocytes *in vivo* dose-dependently and time-dependently, however, the mechanism is not due to apoptosis.

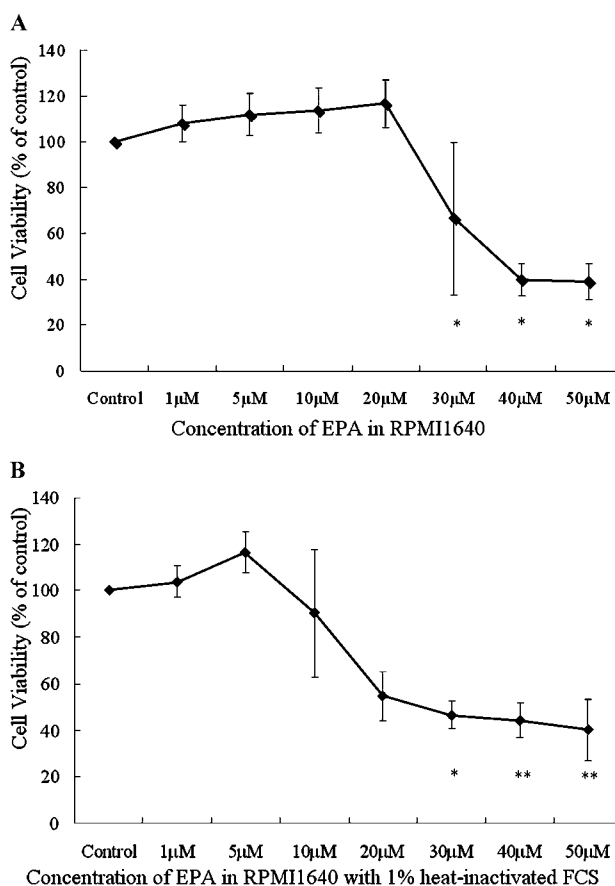


Fig. 1. We seeded synoviocytes (1×10^4 cell/well, $n = 5$) onto 96-well plates of 0–50 μM of EPA and RPMI1640 without FCS and 0–50 μM of EPA (A) or RPMI1640 with 1% incubated FCS (B). After 24 h, we measured cell viabilities by the modified MTT assay (WST-1 assay; Dojindo, Japan). The cell viabilities were decreased at 30 μM , 40 μM , and 50 μM of EPA comparing to 0 μM of EPA (* $p < 0.01$, ** $p < 0.001$). Data was expressed at mean \pm SD.

In animals, omega-3 PUFA has also been found to reduce the severity of experimental cerebral [6] and myocardial infarction [7], to retard autoimmune nephritis and prolong survival of NZB \times NZW F1 mice [8, 9] and reduce the incidence of breast tumors in rats [10]. The anti-inflammatory aspects of omega-3 PUFAs relate to the regulation of prostaglandin and cytokines [11]. EPA is a kind of cell membrane phospholipid as well as arachidonic acid, which is a kind of omega-6 PUFAs [11]. EPA increased PGE3 production and competed with arachidonic acid at the level of incorporation into cell membrane phospholipid, and suppressed the secretion of proinflammatory cytokines (IL-1, TNF- α , IL-6) and COX-2 expression [11]. EPA and COX inhibitor suppressed the expression of Bcl-2 in HL-60 cells, corporately [11]. High concentration of EPA could suppress the proliferation of colon cancer cell, breast cancer cell, and

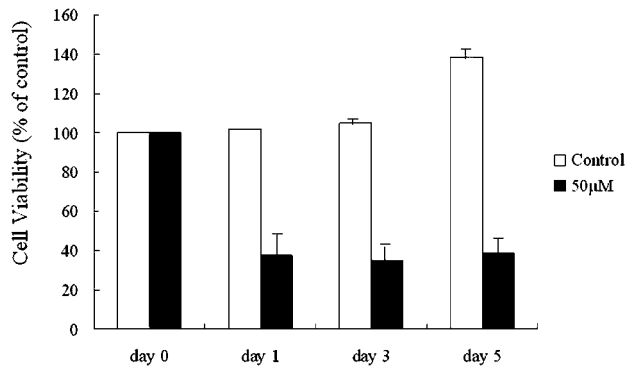


Fig. 2. Synoviocytes (1×10^4 cell/well, $n = 5$) were seeded onto 96-well plates of RPMI 1640 and 1% heat-inactivated FCS with or without 50 μ M EPA. Cell viabilities were measured by the modified MTT assay at day 0, 1, 3, 5. At day 1, 3, 5, cell viabilities were decreased at 50 μ M of EPA comparing to 0 μ M of EPA ($p < 0.001$). Data was expressed at mean \pm SD.

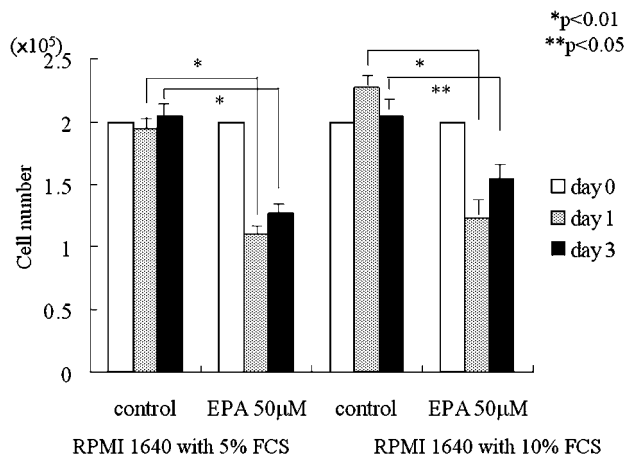


Fig. 3. Synoviocytes (2×10^5 cells/well, $n = 5$) were seeded onto 35 mm diameter plates of RPMI1640 and 5% or 10% heat-inactivated FCS with or without 50 μ M EPA. Floating and adherent synoviocytes were harvested and counted at day 1 and 3. Cell number was decreased at 50 μ M EPA comparing to 0 μ M EPA at both day 1 and day 3 ($*p < 0.01$, $**p < 0.05$). Data represent mean \pm SD.

prostate cancer cell [11]. Moreover, EPA could induce apoptosis in carcinoma cells of malignant lymphoma and leukemia [11]. The pathway of cell death is different on the basis of cell line [11]. Six hours after single intake of 900 mg of EPA, the serum concentration of EPA reaches 153.7 μ g/mL (=485 μ M). EPA exists in normal human body at 33.33 μ g/mL (=105 μ M). When we intake EPA (2700 mg per day, 3 times per day) everyday, the effective serum concentration of EPA reaches 159.79 μ g/mL (=500 μ M). We show that 25 μ M of EPA can suppress the proliferation of

synoviocytes in patients with RA, however, the death pathway of synoviocytes is not through apoptosis. Synovial hyperplasia has been characterized as a tumor-like proliferation and is thought to be a major cause of destruction of cartilage and bone. Increased proliferation and insufficient apoptosis of synovial cell might contribute to its expansion so elimination of proliferating synoviocytes in the rheumatoid synovium seems to be a potentially effective treatment for RA. In this way, EPA is effective for a lot of diseases by regulating the cell cycle and immuno-inflammatory reaction. The mechanism, in particular the suppression of cell growth, is not clarified in detail, however to clarify this matter will lead to new aspects for pathogenesis and therapy for various diseases.

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