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Inhibitory effect of conjugated eicosapentaenoic acid on human DNA topoisomerases I and II

Yuko Yonezawa[a], Tsuyoshi Tsuzuki[b], Takahiro Eitsuka[b], Teruo Miyazawa[b], Takahiko Hada[c], Keisuke Uryu[c], Chikako Murakami-Nakai[a,1], Hiroshi Ikawa[a], Isoko Kuriyama[a], Masaharu Takemura[d], Masahiko Oshige[e], Hiromi Yoshida[a,f], Kengo Sakaguchi[g], Yoshiyuki Mizushina[a,f,*]

[a] *Laboratory of Food and Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan*
[b] *Food and Biodynamic Chemistry Laboratory, Graduate School of Life Science and Agriculture, Tohoku University, Sendai 981-8555, Japan*
[c] *Research and Development Department, Bizen Chemical Co., Ltd., Kumayama-cho, Akaiwa-gun, Okayama 709-0716, Japan*
[d] *Life Science Research Center, Mie University, Tsu, Mie 514-8507, Japan*
[e] *Department of Biochemistry and Molecular Biology, Walther Oncology Center, Indiana University School of Medicine, Indianapolis, IN 46202, USA*
[f] *High Technology Research Center, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan*
[g] *Department of Applied Biological Science, Frontier Research Center for Genomic Drug Discovery, Tokyo University of Science, Noda, Chiba 278-8510, Japan*

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Abstract

DNA topoisomerases (topos) and DNA polymerases (pols) are involved in many aspects of DNA metabolism such as replication reactions. We reported previously that long chain unsaturated fatty acids such as polyunsaturated fatty acids (PUFA) (i.e., eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA)) inhibited the activities of eukaryotic pols in vitro [Biochim. Biophys. Acta 1336 (1997) 509]. In the present study, we found that PUFA also inhibited human topos I and II activities, and the inhibitory effect of conjugated fatty acids converted from EPA and DHA (cEPA and cDHA) on pols and topos was stronger than that of normal EPA and DHA. cEPA and cDHA inhibited the activities of mammalian pols and human topos, but did not affect the activities of plant and prokaryotic pols or other DNA metabolic enzymes tested. cEPA was a stronger inhibitor than cDHA with IC_{50} values for mammalian pols and human topos of 11.0–31.8 and 0.5–2.5 μM, respectively. Therefore, the inhibitory effect of cEPA on topos was stronger than that on pols. Preincubation analysis suggested that cEPA directly bound both topos I and II, but did not bind or interact with substrate DNA. This is the first report that conjugated PUFA such as cEPA act as inhibitors of pols and topos. The results support the therapeutic potential of cEPA as a leading anti-cancer compound that poisons pols and topos.
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Keywords: Conjugated fatty acids; Polyunsaturated fatty acids; Eicosapentaenoic acid; Docosahexanoic acid; DNA polymerases; DNA topoisomerases I and II

Topoisomerases (topos) are key enzymes that control the topological state of DNA. There are two classes of topos: type I enzymes which act by transiently nicking one of the two DNA strands and type II enzymes which nick both DNA strands which are dependent on ATP, and are involved in many vital cellular processes that influence DNA replication, transcription, recombination, integration, and chromosomal segregation [1]. Topo inhibitors are very rare, the most widely studied and characterized being camptothecin, a topo I poison

* Corresponding author. Fax: +81 78 974 5689.
E-mail address: mizushin@nutr.kobegakuin.ac.jp (Y. Mizushina).
1 Present address: Department of Intractable Diseases, International Medical Center of Japan, Shinjuku-ku, Tokyo 162-8655, Japan.

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[2], and etoposide and doxorubicin, topo II poisons [3–7]. In recent years, these enzymes have received special interest because topo inhibitors have emerged as anti-cancer [8] and anti-parasitic agents [9,10].

Topos can be inhibited by two distinct mechanisms, and the inhibitors are divided into two classes accordingly: classes I and II. Class I inhibitors stabilize the enzyme–DNA covalent complex and block the subsequent rejoining of the DNA break. Class II inhibitors, also referred to as catalytic inhibitors, prevent the enzyme and DNA from binding by interacting with either the topo [11,12] or DNA [13].

Eukaryotic cells reportedly contain three replicative DNA polymerases (pols α, δ, σ, and ε), mitochondrial DNA polymerase (pol γ), and at least 13 repair types of DNA polymerases (pols β, δ, ε, ζ, η, θ, κ, λ, σ, φ, pol1-like I, and pol1-like II) [14,15]. Pol catalyzes the addition of deoxyribonucleotides to the 3′-hydroxyl terminus of primed double-stranded DNA molecules [14], and topo catalyzes the concerted breaking and rejoining of DNA strands and is involved in producing the necessary topological and conformational changes in DNA [1,14]. Therefore, pol and topo are critical to many cellular processes such as DNA replication, repair, and recombination, and may act in harmony with each other.

We have screened for inhibitors of pols [16,17], and found that mammalian pols α and β are inhibited by linear-chain fatty acids with the following characteristics: a hydrocarbon chain containing 18 or more carbons, a free carboxyl end, and double bonds with the *cis*-configuration [16,18]. These fatty acids also inhibit the activities of human topos I and II [19]. Fatty acids with conjugated double bonds exist in nature: seaweeds such as red and green algae contain highly unsaturated conjugated fatty acids, i.e., conjugated EPA (cEPA; 5*Z*7*E*9*E*14*Z*17*Z*-20:5), bosseopentaenoic acid (5*Z*8*Z*10*E*12*E*14*Z*-20:5), and stellaheptaenoic acid (4*Z*7*Z*9*E*11*E*13*Z*16*Z*19*Z*-22:7) [20,21]. We realized the importance of the two classes of polyunsaturated fatty acids (PUFA)[2]; eicosapentaenoic acid (EPA; 5*Z*8*Z*11*Z*14*Z*17*Z*-20:5) and docosahexanoic acid (DHA; 4*Z*7*Z*10*Z*13*Z*16*Z*19*Z*-22:6), normal and conjugated. Thus, conjugated PUFA seem to be an ideal model for the study not only of the molecular mechanisms that inhibit pol and topo activities for the development of new anticancer drugs, but also of cellular processes such as DNA replication, repair, and recombination.

In this study, we determined the mechanisms of the inhibitory effect of cEPA/EPA on pols and topos in vitro.

[2] *Abbreviations used:* cEPA, conjugated eicosapentaenoic acid; EPA, eicosapentaenoic acid; cDHA, conjugated docosahexanoic acid; DHA, docosahexanoic acid; PUFA, polyunsaturated fatty acids; topo, topoisomerase; pol, DNA polymerase; DMSO, dimethyl sulfoxide; dsDNA, double-stranded DNA; EtBr, ethidium bromide; SM, supercoiled monomer; NM, nicked monomer; RM, relaxed monomer; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

Materials and methods

Materials

Polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), were purchased from Nu-Chek-Prep (Minnesota, USA). Nucleotides such as [^{3}H]-2′-deoxythymidine 5′-triphosphate (dTTP) (43 Ci/mmol), chemically synthesized template-primers such as poly(dA) and oligo(dT)$_{12-18}$, and calf thymus double-stranded DNA (dsDNA) were obtained from Amersham Biosciences (Buckinghamshire, UK). Supercoiled pBR322 was bought from Takara (Tokyo, Japan). Kinetoplast DNA and rabbit anti-topo II antibody were obtained from TopoGen (Columbus, OH, USA). Alkaline phosphatase conjugated forms of goat anti-rabbit IgG was purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade and purchased from Nacalai Tesque (Kyoto, Japan).

Preparation of conjugated EPA and DHA by alkaline treatment

Conjugated EPA and DHA were prepared by alkaline treatment following the AOAC method with slight modifications [22]. Potassium hydroxide at a concentration of 6.6 or 21% (w/w) in ethylene glycol was prepared and the KOH solution was bubbled for 5 min with nitrogen gas. Ten milligrams of EPA or DHA was added to 1 ml of the 6.6 or 21% KOH solution in a test tube (10 ml volume). The mixture was bubbled with nitrogen gas and then screw-capped and allowed to stand for 5 or 10 min at 180 °C. The reaction mixture was cooled and 1 ml methanol was added. The mixture was acidified to below pH 2 with 2 ml of 6 N HCl. After dilution with 2 ml of distilled water, the conjugated fatty acid was extracted with 5 ml of hexane. The hexane extract was then washed with 3 ml of 30% methanol and with 3 ml of distilled water before being evaporated under a nitrogen gas stream. The conjugated fatty acids were stored at −20 °C after being purged with nitrogen gas. UV/VIS spectrophotometric analysis of the conjugated fatty acid was performed with a Shimadzu UV-2400PC. Spectrophotometric readings confirmed the conjugation of fatty acids of pentaene (345 nm) and hexaene (375 nm) [22,23]. The conjugated PUFA and normal PUFA were dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s.

Enzymes

DNA polymerase α (pol α) was purified from calf thymus by immuno-affinity column chromatography as described previously [24]. Recombinant rat pol β was purified from *Escherichia coli* JMpβ5 as described by Date et al. [25]. The human pol γ catalytic gene was

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