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Effect of conjugated linoleic acid mixture supplemented daily after carcinogen application on linoleic and arachidonic acid metabolites in rat serum and induced tumours

Małgorzata Jelińska [a,*], Agnieszka Białek [a], Hanna Mojska [b], Iwona Gielecińska [b], Andrzej Tokarz [a]

[a] Department of Bromatology, Faculty of Pharmacy with the Laboratory Medicine Division, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
[b] Department of Food and Food Supplements, National Food and Nutrition Institute, Powsińska 61/63, 02-903 Warsaw, Poland

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ABSTRACT

Conjugated linoleic acid (CLA) is thought to exert anticarcinogenic, antiatherogenic, anti-inflammatory and weight loss effects. The impact on eicosanoid biosynthesis may be one of the mechanisms of its action. The aim of this study was to establish whether CLA mixture supplemented daily after administration of carcinogen (7, 12-dimethylbenz[a]anthracene, DMBA) influenced the concentration of linoleic and arachidonic acid metabolites: 13- or 9-hydroxyoctadecadienoic acids (13-, 9-HODE) and 15-, 12- or 5-hydroxyeicosatetraenoic acids (15-, 12- or 5-HETE) and prostaglandin E_2 (PGE_2) in rat serum and DMBA-induced tumours. The correlations between polyunsaturated fatty acids (PUFA) and HETE and HODE contents in serum were also investigated.
Female Sprague–Dawley rats divided into three groups according to the diet (1% Bio-C.L.A., 2% Bio-C.L.A. and plant oil in the control group) were used in the study. On the 50th day of life some of the animals in every dietary group were administered DMBA to induce tumours. Since that day, the rats were fed one of the above-mentioned diets. After 15 weeks the animals were sacrificed and blood and tumours were collected. HETE and HODE were extracted using a solid-phase extraction (SPE) method on C18 columns and analysed with LC-MS/MS.
The results of our study showed that CLA daily supplementation after carcinogen administration influence LA and AA metabolite levels in serum and tumours. However, the ratios of eicosanoids having opposite effects (e.g. 12-HETE/15-HETE), not concentrations of particular compounds, appear to be better indicators of pathological processes.

1. Introduction

Conjugated linoleic acid (CLA) is a group of naturally occurring fatty acids synthesised from linoleic acid by bacteria present in alimentary tract of ruminant animals or as a result of endogenous conversion of transvaccenic acid by Δ9-desaturase in tissues, especially the mammary glands [1]. CLA may be also synthetically produced by partial hydrogenation or alkali isomerisation of linoleic acid or oils rich in linoleic acid (e.g. sunflower or safflower oils) [2,3]. That pathway is used for the industrial production of commonly available CLA preparations. CLA is a mixture of positional and geometric isomers of linoleic acid (LA) with double bonds between carbon atoms 7 and 9, 8 and 10, 9 and 11, 10 and 12 or 11 and 13. They occur in a cis or trans configuration. The most prevalent form is the cis-9,trans-11 CLA isomer found in ruminant-derived foods like milk, cheese and meat [4]. The other active isomer is trans-10,cis-12 CLA, present only in trace amounts in animal-derived foods. However, it was found in equal proportions with cis-9, trans-11 CLA in commercial CLA supplements [4]. As Yu et al. indicated, these supplements may contain different concentrations of total CLA, its two main active isomers and fatty acid profile [5]. That may be due to various compositions of fatty acids in the source plant oil used to CLA synthesis and to conditions of isomerisation reactions [5].

CLA is thought to exert beneficial effects in atherosclerosis prevention [6], obesity reduction and cancer prevention [7,8]. Presently, the mechanisms of its anti-carcinogenic action are being researched. An impact of CLA on eicosanoid biosynthesis is thought to be one of them.

Eicosanoids are local, biologically active metabolites of 20-carbon polyunsaturated fatty acids — arachidonic acid (AA), dihomo-gamma-linolenic acid (DGLA), and eicosapentaenoic acid (EPA). They are synthesised by their oxidation by cyclooxygenase (COX) and lipoxygenases (LOX). Arachidonic acid derivatives synthesised by COX pathway belong to the best characterised compounds. They

Abbreviations: AA, arachidonic acid; CLA, conjugated linoleic acid; COX, cyclooxygenase; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DMBA, 7,12-dimethylbenz[a]anthracene; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; LA, linoleic acid; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LOX, lipoxygenase; PUFA, polyunsaturated fatty acids; SEM, standard error of mean; TXA_2, thromboxane A_2

* Corresponding author.
E-mail addresses: malgorzata.jelinska@wum.edu.pl (M. Jelińska), agnieszka.bialek@wum.edu.pl (A. Białek), hmojska@izz.waw.pl (H. Mojska), gielecin@izz.waw.pl (I. Gielecińska), andrzej.tokarz@wum.edu.pl (A. Tokarz).

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include prostaglandin E_2 (PGE_2), prostacyclin or thromboxane A_2 (TXA_2). PGE_2 appears to be a pro-inflammatory mediator and was synthesised by some tumours [9]. Its elevated levels were noted in tumours of digestive tract and in blood collected from vessels draining a tumour area [10]. PGE_2 production correlated with fatty acid content in the diet and increased in animals fed a diet rich in linoleic acid. In contrast to COX metabolites, hydroxyeicosatetraenoic acids (HETE), synthesised from arachidonic acid by LOX were initially considered unimportant compounds without biological activity. Since different LOX isoforms are involved in arachidonic acid metabolism, three main isomers of HETE are generated: 5-, 12- and 15-hydroxyeicosatetraenoic acids (5-, 12-and 15-HETE). Nowadays they are thought to participate in a number of pathological processes, such as inflammation, atherosclerosis, hypertension or cancer [11]. 5- and 12-HETE are involved in tumour development [12]. 12-HETE may enhance tumour cell adhesion to endothelium, stimulate their proliferation, motility and angiogenesis and as a result plays a critical role in metastasis [13]. Exogenous 5-HETE stimulates the proliferation and growth of prostate, breast and lung cancer cells and acts as a survival factor [11,14]. The inhibition of its synthesis by nordihydroguaiaretic acid (NDGA), AA-861, MK-886 and Zileuton, which are 5-LOX inhibitors, resulted in decreased proliferation and enhanced apoptosis of cancer cells. Contrary to pro-cancerogenic 5- and 12-HETE, 15-HETE appears to have protective and anti-tumourigenic activity. It activates peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear transcription factor involved in epithelial differentiation and the arrest of cell growth. As a result, 15-HETE inhibits proliferation and induces apoptosis of prostate carcinoma or colorectal cancer cells [15,16]. Besides 15-HETE, 13-hydroxyoctadecadienoic acid (13-HODE), linoleic acid (LA) metabolite synthesised by 15-LOX-1, is another fatty acid derivative exerting protective and anti-tumourigenic role. Studies on rat skin or colon tumours indicate that 13-HODE may inhibit proliferation and induces apoptosis [17]. Another linoleic acid derivative, 9-hydroxyoctadecadienoic acid (9-HODE), produced on the 5-LOX pathway was described to stimulate cell proliferation [18]. Despite developing knowledge of various fatty acid LOX metabolite activities, there is still little information on the impact of diet and dietary fat on them, especially in tumour conditions. Fish oil containing diet significantly decreased 12-HETE, 15-HETE and 13-HODE levels in azoxymethane-induced rat colon tumours, comparing to animals consuming corn oil containing diet [19]. That correlated with enhanced apoptotic index in the fish oil containing group. Similar observation was made by Rose et al. who described significant reduction of arachidonic acid level as well as its LOX metabolites, 12- and 15-HETE, in breast tumours developed in mice fed EPA or DHA containing diet, when compared to linoleic acid high diet [20]. The authors explained inhibitory effects of dietary fish oil on breast cancer growth and metastasis with mechanisms that probably involved suppression of tumour eicosanoid biosynthesis. Ten-week supplementation of the human diet with fish oil has been shown to result in decreased production of 5-HETE and leukotriene B4 by inflammatory cells [21]. In another experiment Espada et al. observed that chia oil rich in n-3 fatty acids (63% α-linolenic acid and 21% linoleic acid) decreased 12-HETE level in murine mammary gland adenocarcinomas, compared to safflower oil (76% linoleic acid and 0.24% α-linolenic acid) and control diet (12% linoleic acid and 3.5% α-linolenic acid) in mice [22]. Ramsden et al. noted that lower dietary linoleic acid levels reduced the concentration of 13- and 9-HODE [23]. It was also observed that in breast cancer cells, trans-10,cis-12 CLA decreased cell growth and the production of hydroxyeicosatetraenoic acid (5-HETE), which is an arachidonic acid metabolite, synthesised by 5-lipoxygenase (5-LOX) [24].

The aim of this study was to establish if commercially available in pharmacies or health food stores conjugated linoleic acids mixture (cis-9,trans-11 isomer and trans-10,cis-12 isomer in equal proportions), supplemented daily after carcinogenic agent administration influenced the concentration of linoleic and arachidonic acid derivatives in rat serum and 7,12-dimethylbenz[a]anthracene (DMBA)-induced tumours. The correlations between polyunsaturated fatty acids (PUFA) and HETE contents in serum were also investigated.

2. Materials and methods

2.1. Standards and chemicals

Eicosanoid standards: 15-hydroxyeicosatetraenoic acid (15-HETE), 12-hydroxyeicosatetraenoic acid (12-HETE), 5-hydroxyeicosatetraenoic acid (5-HETE), 13-hydroxyoctadecadienoic acid (13-HODE), 9-hydroxyoctadecadienoic acid (9-HODE) and prostaglandin E_2 (PGE_2) were purchased from Cayman Chemical Company, USA. LC-MS grade methanol, acetonitrile, ethanol and solid-phase extraction (SPE) cartridges (Bakerbond C18, 500 mg/3 mL) were purchased from J.T. Baker. Formic acid and 7,12-dimethylbenz[a]anthracene (DMBA) were purchased from Sigma-Aldrich. Deionised water was purified on the water purification system (Direct Q, Millipore). Bio-C.L.A. was obtained from Pharma-Nord, Denmark.

2.2. Animals and experiment

Female Sprague–Dawley rats (n = 50), purchased from Division of Experimental Animals, Department of General and Experimental Pathology (Medical University of Warsaw, Poland), were used in the study. The animals (30 days old) were fed the rat standard diet (Labofeed H, Feed and Concentrates Production Plant, A. Morawski, Kcynia, Poland) ad libitum. The diet was composed of the following compounds (per 1 kg): protein (222 g), fat (50 g), fibre (45 g), ash (60 g), carbohydrates (500 g), vitamin A (15,000 IU), vitamin D3 (1000 IU), vitamin E (90 mg), vitamin K3 (3 mg), vitamin B1 (21 mg), vitamin B2 (16 mg), vitamin B6 (17 mg), vitamin B12 (80 μg), pantothenic acid (30 mg), folic acid (5 mg), nicotinic acid (133 mg), Ca (9.5 g), P (7.7 g), Mg (3 g), K (10 g), Na (2 g), Cl (2.5 g), S (1.9 g), Fe (170 mg), Mn (68 mg), Zn (78 mg), Cu (16 mg), Co (0.3 mg), I (0.2 mg), and Se (0.4 mg). Linoleic acid was the main fatty acid in the rat chow. Its concentration was about 40%, followed by α-linolenic acid (22%), oleic acid (16%), palmitic acid (13%) and stearic acid (3%). Other fatty acids were in trace concentrations. Since the 50th day of life the rats were given intragastrically 0.15 mL/day of oil rich in linoleic acid (groups A1 and G1) or Bio-C.L.A. (Pharma-Nord, Denmark) (other groups) in two concentrations. Groups B1 and D1 were given 0.15 mL/day of Bio-C.L.A., which corresponded with 1% content in a diet, whereas groups C1 and E1 were given 0.30 mL/day, equal to 2% CLA in a diet [25]. Dietary groups numbered 8 (A1, G1, D1 and E1) or 9 (B1 and C1) animals. Fatty acid profiles (%) in administered oils are introduced in Fig. 1. When 50 days old, the animals from groups A1, B1 and C1 were administered intragastrically a single dose (80 mg/kg body weight) of carcinogenic agent — 7,12-dimethylbenz[a]anthracene (DMBA, Sigma-Aldrich) to induce tumours. Fifteen weeks after DMBA administration, the rats were decapitated. Blood and tumours were collected. Serum was obtained by centrifugation of blood at 4 °C at 3000 rpm. Serum and tumours were stored at −20 °C until further analyses.

The experiment was approved by The Ethical Committee on Animal Experiments at the Medical University of Warsaw.

2.3. HETE and HODE determination

2.3.1. Sample preparation

Methanol (0.5 mL) was added to serum samples (0.4 mL), followed by water (4 mL), so as to obtain approximately 10% methanol. Tumour samples (approximately 0.2 g) were homogenised in water (2 mL). During that action the homogeniser was kept in crushed ice. The samples were incubated at 4 °C for 30 min then tumour samples were centrifugated at 3000 rpm for 5 min to remove precipitated proteins. Tumour supernatants and serum samples were loaded onto SPE