Prostaglandins & other Lipid Mediators 116–117 (2015) 10–18

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Original Research Article

Influence of maternal diet enrichment with conjugated linoleic acids on lipoxygenase metabolites of polyunsaturated fatty acids in serum of their offspring with 7,12-dimethylbenz[a]anthracene induced mammary tumors

Agnieszka Białek*, Małgorzata Jelińska, Andrzej Tokarz

Department of Bromatology, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland

ARTICLE INFO

Article history:
Received 23 June 2014
Received in revised form 2 October 2014
Accepted 6 October 2014
Available online 16 October 2014

Keywords:
Breast cancer
Conjugated linoleic acids
HEPE
HETE
HODE
Lipoxygenase

ABSTRACT

Conjugated linoleic acids (CLA), which are a group of naturally occurring in food isomers of linoleic acid, seem to be active in each step of cancer development. There are many possible mechanisms of this action, and interactions with polyunsaturated fatty acids (PUFA) in lipoxygenase (LOX) and cyclooxygenase (COX) pathways are among the most likely ones.

The aim of this study was to assess the influence of diet supplementation with CLA of pregnant and breastfeeding Sprague-Dawley female rats on selected polyunsaturated fatty acids and their LOX metabolites concentrations in serum of the progeny with chemically induced mammary tumors.

We confirmed that higher supply of CLA in the diet of female rats corresponded with the lower susceptibility to chemically induced mammary tumors in their female offspring. It also influenced the polyunsaturated n-3 and n-6 fatty acid concentrations in serum, as well as the concentrations of their LOX metabolites. The significant negative correlation between the concentrations of two CLA isomers in serum and linoleic acid ($p = 0.0144$, $p = 0.0098$), eicosapentaenoic acid ($p = 0.0158$, $p = 0.0124$), and 5-HEPE ($p = 0.0014$, $p = 0.01690$) and between cis-9, trans-11 CLA and 15-HEPE was detected, whereas arachidonic acid concentration positively correlated with CLA concentration in serum ($p = 0.0150$, $p = 0.0231$).

Our results indicate that CLA can compete with PUFA and influence serum concentration of PUFA and their LOX metabolites, which could partly explain the anticancerogenic action of CLA.

Introduction

Breast cancer is the most frequent type of cancer among women in global population [1]. Although the etiology of this disease is unknown [1,2], many nutritional factors are associated with elevated or reduced risk of this type of cancer [1,3]. The quantity and quality of fat and fatty acid ratio in a diet are especially important. Conjugated linoleic acids (CLA) are also often investigated because of their numerous health-promoting properties.

CLA are positional and geometric isomers of linoleic acid with two conjugated double bonds in their carbon chains. They are natural components of food products such as milk, dairy products and ruminant meat. Cis-9, trans-11 octadecadienoic acid (rumenic acid – RA) is the predominant CLA isomer in dietary products. It constitutes over 90% of all CLA isomers [4], whereas the second important CLA isomer: trans-10, cis-12 octadecadienoic acid is present mainly in dietary supplements [5]. Dietary intake is the main source of CLA for humans, despite the fact that rumenic acid can be endogenously synthesized from trans-11 octadecenoic acid (vaccenic acid – VA) by the action of Δ^9-desaturase [6]. CLA can exert the positive influence in different pathological conditions, such as atherosclerosis, diabetes, obesity and different types of cancer [7]. CLA not only can reduce the cancerous process risk but also influence each step of breast cancer development, from the initiation to metastasis [8].

Abbreviations: AA, arachidonic acid; ALA, α-linoleic acid; CLA, conjugated linoleic acids; DHA, docosahexaenoic acid; DMBA, 7,12-dimethylbenz[a]anthracene; EPA, eicosapentaenoic acid; FA, fatty acids; FAME, fatty acids methyl esters; GC, gas chromatography; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HPLC, high performance liquid chromatography; LA, linoleic acid; OL, oleic acid; PGE_2, prostaglandin E_2; PPARγ, peroxisome proliferator-activated receptor gamma; PUFA, polyunsaturated fatty acids; RA, rumenic acid; SPE, solid phase extraction; TEB, terminal end buds; VA, vaccenic acid.

* Corresponding author. Tel.: +48 22 5720745; fax: +48 22 5720785.
E-mail addresses: agnieszka.bialek@wum.edu.pl (A. Białek), malgorzata.jelinska@wum.edu.pl (M. Jelińska), andrzej.tokarz@wum.edu.pl (A. Tokarz).

http://dx.doi.org/10.1016/j.prostaglandins.2014.10.001
1098-8823/

There are many possible mechanisms of their action, which are still investigated, but the competition with polyunsaturated n-3 and n-6 fatty acids and the impact on their metabolism are often highlighted.

Eicosanoids are polyunsaturated fatty acids (PUFA) metabolites whose body concentrations often depend on precursor intake. Besides well-studied prostaglandin E_2 (PGE_2), synthesized from arachidonic acid (AA) on the cyclooxygenase (COX) pathway, lipoxygenase (LOX) metabolites of that fatty acid appear to a play role in various pathological states, such as carcinogenesis. 12- and 5-hydroxyeicosatetraenoic acids (12-HETE, 5-HETE) were observed to stimulate cancer cell proliferation, motility and angiogenesis, as well as inhibit apoptosis [9,10]. All these lead to cancer development and metastasis [11]. Another arachidonic acid derivative, 15-hydroxyeicosatetraenoic acid (15-HETE), is thought to have opposite effect in many types of cancer, activating peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear transcription factor involved in epithelial differentiation and the arrest of cell growth [12,13]. The same lipoxygenases convert eicosapentaenoic acid (EPA) to similar metabolites – 15-, 12- and 5-hydroxyeicosapentaenoic acids (15-, 12- and 5-HEPE). Linoleic acid (LA) metabolites 13- and 9-hydroxyoctadecadienoic acids (13-HODE and 9-HODE) also play a role in tumorigenesis. 13-HODE isomer was observed to inhibit cell growth and induce apoptosis in the breast cancer cell lines, acting through down-regulating PPAR-γ [14]. Results of numerous researches suggest that CLA affect the COX pathway of fatty acids metabolism. In macrophages cultured with CLA reduction of thromboxane A2 and PGE_2 concentration and significant change in COX-2 expression as well as COX-1 inhibition resulting from competition of CLA and linoleic acid with arachidonic acid were observed [15]. CLA also repress AP-1-mediated activation of COX-2 transcription [16], suppress the expression of COX-2 mRNA [17] and regulate EP2 protein expression [18]. However, their influence on LOX pathway of fatty acids metabolism is still under investigation.

According to World Health Organization appropriate feeding practices (especially breastfeeding) play a significant role in improving the health and nutrition of young children, and they also confer significant long-term benefits during adolescence and adulthood [19]. The aim of this study was to assess the influence of diet supplementation of pregnant and breastfeeding female Sprague-Dawley rats with conjugated linoleic acids on polyunsaturated fatty acids profile and on their lipoxygenase metabolites concentrations (hydroxyeicosatetraenoic acids, HETE; hydroxyoctadecadienoic acids, HODE; hydroxyeicosapentaenoic acids, HEPE) in serum and tumors of the progeny with chemically induced mammary tumors.

Materials and methods

Animals

The Local Ethical Committee on Animal Experiments approved the guiding principles in the care and use of laboratory animals in this research. Maiden adult female Sprague–Dawley rats ($n = 8$) were purchased from Division of Experimental Animals, Department of General and Experimental Pathology (Medical University of Warsaw, Warsaw, Poland). They were housed for 1-week adaptation period and the whole experiment in animal room in stainless steel cages under controlled conditions: 21 °C, in a 12 h light: 12 h dark cycle. They were fed the standard fodder Labofeed H (Feed and Concentrates Production Plant, A. Morawski, Żurawia 19, Kcynia, Poland) and drinking water ad libitum. The Labofeed H fodder contained: protein (22.0%), fat (4.0%), starch (30.0%), fiber (5.0%) and minerals (6.5%). The animals, after the adaptation period, were randomly divided into two groups of different dietary supplementation. Group CLA received Bio-C.L.A. (Pharma Nord Denmark) whereas group VOL was supplemented with vegetable oil given intragastrically in the amount 0.15 mL/day. This oil was purchased from the manufacturer of the CLA preparation and it did not contain CLA isomers. After 1 week female Sprague-Dawley rats were mated with males from the same species. Males' diet was not modified. Females' diet modification lasted for the whole period of pregnancy and breastfeeding of the progeny. The offspring were separated from their mothers at the 30th day of life. In the experiment only female offspring were used. Within the groups of supplementation they were divided into two subgroups of 8–10 individuals each. The first subgroup's diet was enriched with the same dietary supplement that had been previously given to their mothers (group CLA (M+/P+) received Bio-C.L.A. (Pharma Nord Denmark) and VOL (M+/P+) – vegetable oil), and the second subgroup was fed the standard Labofeed H fodder (groups CLA (M+/P−) and VOL (M+/P−)). Fig. 1 shows the fatty acids composition of applied diets. Dietary supplementation of subgroups was conducted for the following 21 weeks counting from the 30th day of life of the progeny. At the 50th day of life all descendants were given via gavage a single dose of 80 mg/kg body weight of carcinogenic agent – DMBA (7,12-Dimethylbenz[a]anthracene, approx. 95%, Sigma–Aldrich) for the induction of mammary tumors. The progeny was weekly weighed and palpated to detect the appearance of mammary tumors. They

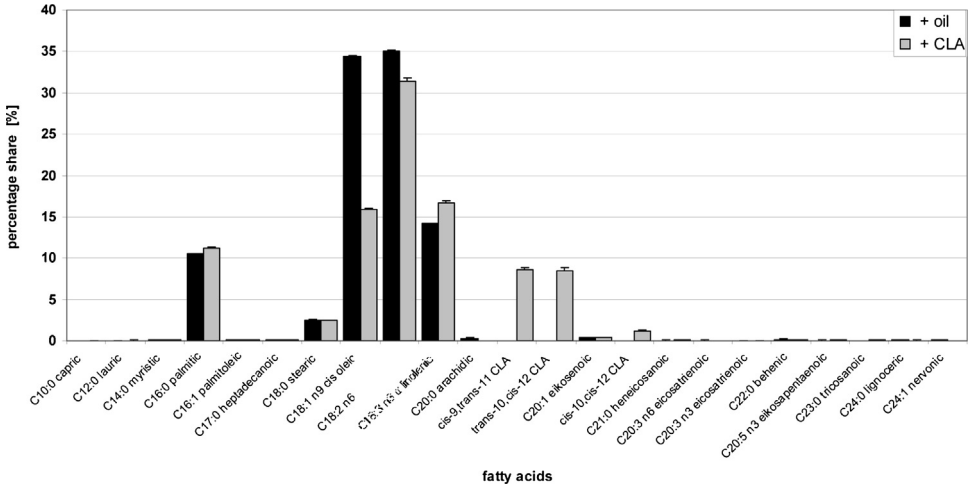

Fig. 1. Fatty acids composition of applied diets. Figure presents the percentage share of each individual fatty acid in the total pool of all fatty acids (%)