Prostaglandins, Leukotrienes and Essential Fatty Acids 106 (2016) 1–10

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Review

Chemometric analysis of the interactions among different parameters describing health conditions, breast cancer risk and fatty acids profile in serum of rats supplemented with conjugated linoleic acids

Agnieszka Białek [a,*,1], Paweł Zagrodzki [b,c,1], Andrzej Tokarz [a,1]

[a] Department of Bromatology, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
[b] Department of Food Chemistry and Nutrition, Jagiellonian University Medical College, Medyczna 9, 30-688 Cracow, Poland
[c] H. Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, Radzikowskiego 152, 31-342 Cracow, Poland

ARTICLE INFO

Article history:
Received 16 July 2015
Received in revised form
15 October 2015
Accepted 14 December 2015

Keywords:
Fatty acids
Breast cancer
PARTIAL Least Square
Cluster analysis

ABSTRACT

We investigated how different doses of conjugated linoleic acids applied for various periods of time influence breast cancer risk and fatty acids profile in serum of rats treated or not with 7,12-dimethylbenz [a]anthracene (DMBA). We also search for interactions among parameters describing health conditions and cancer risk.

Animals were divided into 18 groups with different diet modifications (vegetable oil, 1.0%, 2.0% additions of CLA) and different periods of supplementation. In groups treated with DMBA mammary adenocarcinomas appeared. Due to the complexity of experiment apart from statistical analysis a chemometric tool—Partial Least Square method was applied. Analysis of pairs of correlated parameters allowed to identify some regularities concerning the relationships between fatty acid profiles and clinical features of animals. Fatty acids profile was the result of prolonged exposure to high dose of CLA and DMBA administration. These two factors underlined the differences in fatty acids profiles among clusters of animals.

1. Introduction

Conjugated fatty acids is a term used to refer to a group of polyunsaturated fatty acids with conjugated double bonds system in their carbon chains. Conjugated linoleic acids (CLA) are a group of positional and geometric isomers of linoleic acid (C18:2, n-6, LA). The natural sources of CLA are milk, dairy products and meat of ruminants, where they are built in triacylglycerols (TAG). CLA are present also in numerous anti-obesity dietary supplements where they are obtained from vegetable oils rich in linoleic acid by alkaline isomerisation and they are in the form of free fatty acids. However, there are no differences in digestibility of *cis*-9, *trans*-11 CLA (rumenic acid, RA) in rats, irrespective to its chemical form. *Cis*-9, *trans*-11 CLA is completely absorbed from gastrointestinal tract [1]. Moreover Tsuzuki et al. found no differences in bioavailability in rats between two major CLA isomers: *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA both as TAG and as free fatty acids [2]. Results of numerous experiments conducted since 1970s proved health promoting properties of CLA in many pathological conditions, e.g. obesity, arteriosclerosis, cardiovascular diseases, osteoporosis, diabetes, insulin resistance, inflammation and different types of cancer, but precise mechanisms of their action are still under investigation. Many researchers emphasize great importance of CLA in modification of the cancerous process risk, especially within mammary glands, but their action depends on the conformation of the isomer [3].

Models of chemically induced carcinogenesis in rats are commonly used in many experiments concerning formation and progress of tumours, and also when the preventive properties of diet components are investigated. 7,12-dimethylbenz[a]anthracene (DMBA) is one of the commonly used carcinogenic factors. Its single application into the stomach is very effective in mammary tumours formation in young female Sprague-Dawley rats, while mainly its metabolites form the DNA adducts in proliferating cells

Abbreviations: AA, arachidonic acid; ALA, α-linoleic acid; CA, cluster analysis; CLA, conjugated linoleic acids; DHA, docosahexaenoic acid; DMBA, 7,12-dimethylbenz[a]anthracene; EPA, eicosapentaenoic acid; ER, α-oestrogenic receptors α; FA, fatty acids; FAME, fatty acids methyl esters; GC, gas chromatography; GLA, γ-linolenic acid; LA, linoleic acid; OL, oleic acid; PLS, Partial Least Square; RA, rumenic acid; TAG, triacylglycerols; VA, vaccenic acid

* Corresponding author.
E-mail addresses: agnieszka.bialek@wum.edu.pl (A. Białek), Pawel.Zagrodzki@ifj.edu.pl (P. Zagrodzki), andrzej.tokarz@wum.edu.pl (A. Tokarz).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plefa.2015.12.006
0952-3278/

of terminal end buds and interact in neoplastic transformation [4]. Active metabolites of DMBA arising in liver and mammary glands bind with DNA and activate protooncogens, as well as bind with different types of proteins and interact with numerous cellular processes [5]. Effectiveness of cancer induction with DMBA depends on hormones' level [6] especially estrogens [4] and also on the response of immune system [6]. According to Ma et al. all mammary tumours induced by DMBA administration contained estrogenic receptors α (ER α) [7]. We previously observed significantly reduced risk of breast cancer appearance caused by CLA presence in diet using this model of carcinogenesis and [8–10] proposed some possible mechanism concerning CLA action [10–13]. We showed that CLA higher supply in mothers' diet during pregnancy and breastfeeding caused their incorporation into tissues of children and also exerts health-promoting effect in offspring's adult life by decreasing the breast cancer risk [9,13].

It seems that applied dose of CLA, duration of diet supplementation and moment of life can influence the CLA impact on health conditions. In present study we wanted to investigate how diet supplementation with different doses of CLA applied for various periods of time influences the breast cancer risk and fatty acids profile in serum of female Sprague-Dawley rats subjected for carcinogenic agent administration. We also search for possible interactions among fatty acids profile in serum and different parameters describing health conditions and cancer risk in rats. Due to the complexity of conducted experiment and a large amount of obtained data, apart from statistical tests we tried to use some chemometric tools to interpret our results. Cluster analysis (CA) was already applied in our former approach [14], and now Partial Least Square (PLS) method was chosen for further analysis of complex data.

2. Materials and methods

2.1. Animals

The Local Ethical Committee on Animal Experiments approved the whole experiment and guiding principles in the use and care of laboratory animals. Female Sprague-Dawley rats ($n=172$, age—30 days) were purchased from Division of Experimental Animals, Department of General and Experimental Pathology (Medical University of Warsaw, Warsaw, Poland). During whole research animals were kept in animal room at 21 °C, in a 12 h light: 12 h dark cycle and they were fed *ad libitum* a standard laboratory fodder Labofeed H (Feed and Concentrates Production Plant, A. Morawski, Żurawia 19, Kcynia, Poland) and water. Labofeed H is composed of 22.0% protein, 4.0% fat, 30.0% starch, 5.0% fibre, 6.5% minerals. Detailed composition of applied fodder is presented in Table 1.

After 1-week adaptation animals were randomly divided into 18 groups (A1–G3) with different dietary supplementation and carcinogenic agent treatment. Diet of B and C groups was supplemented with conjugated linoleic acid (Bio-C.L.A. (Pharma Nord Denmark)), given via gavage in the amount of 0.15 ml/day (B) or 0.30 ml/day (C) after (B1, C1), before (B2, C2), and before and after (B3, C3) carcinogenic agent—(7,12-dimethylbenz[a]anthracene, approx. 95%, Sigma-Aldrich) administration. Rats received single dose of 80 mg/kg body weight of DMBA intragastrically at 50th day of life. Groups D and E, without chemical induction of carcinogenesis, received CLA in the amount of 0.15 ml/day (D) or 0.30 ml/day (E) from 50th day of life till decapitation (D1, E1), from 37th to 50th day of life (D2, E2) or from 37th day of life till decapitation (D3, E3). Groups labelled with letter "A" received intragastrically vegetable oil in the amount of 0.15 ml/day after (A1), before (A2) or before and after (A3) DMBA administration at 50th day of life. Control groups "G" were supplemented with vegetable oil from 50th day of life till decapitation (G1), from 37th to 50th day of life (G2) or from 37th day of life till decapitation (G3). Vegetable oil, which did not contain conjugated linoleic acids, was purchased from Pharma Nord Denmark, where it was used as the substrate to CLA synthesis. Table 2 shows the fatty acid composition of applied supplements. During the entire experiment, which lasted for the following 21 weeks, the rats were weighed weekly and palpated to detect the appearance of tumours. All animals were decapitated and exsanguinated in the 21st week of the experiment except the groups long-term supplemented with higher doses of CLA (C1, C3, E1, E3), which were decapitated in the 15th week of the experiment, because of the much lower body weight and cachexia. There were no spontaneous tumours during the experiment in groups of rats without DMBA treatment. The effectiveness of mammary cancers induction was determined as the percentage of animals with tumours in each group. Tumours were identified as adenocarcinomas of mammary gland, as previously described [8].

Table 1
Composition of Labofeed H fodder.

Protein [g]	210.0		
Fat [g]	39.2		
Fibre [g]	43.2		
Strach [g]	300.0		
Ash [g]	55.0		
Vitamin A [IU]	15,000	Lysine [g]	14.5
Vitamin D_3 [IU]	1000	Methionine [g]	4.1
Vitamin E [mg]	90.0	Tryptophan [g]	3.0
Vitamin K_3 [mg]	3.0	Threonine [g]	7.4
Vitamin B_1 [mg]	21.0	Isoleucine [g]	17.5
Vitamin B_2 [mg]	16.0	Valine [g]	11.0
Vitamin B_6 [mg]	17.0	Histidine [g]	6.0
Vitamin B_{12} [μg]	80.0	Arginine [g]	13.0
Pantothenate [mg]	30.0	Phenylalanine [g]	10.0
Folic acid [mg]	5.0	Tyrosine [g]	7.8
Nicotinic acid [mg]	133.0	Choline [mg]	2750.0
Biotin [mg]	0.4		
Calcium [g]	10.0	Iron [mg]	250.0
Phosphorus total [g]	8.17	Manganese [mg]	100.0
Phosphorus saturated [g]	4.5	Zinc [mg]	76.9
Magnesium [g]	3.0	Copper [mg]	21.3
Potassium [g]	9.4	Cpbalt [mg]	2.0
Sodium [g]	2.2	Iodine [mg]	1.0
Chlorine [g]	2.5	Selenium [mg]	0.5
Sulphur [g]	1.9		

Declared data are expressed per kg of diet.

Table 2
Fatty acids profile of applied supplements.

Fatty acid	Bio-C.L.A.	Oil
C16:0	4.6 ± 0.0	5.7 ± 0.0
C18:0	2.2 ± 0.0	0.4 ± 0.0
C18:1 n-9 *cis* OL	11.0 ± 0.0	64.5 ± 0.1
C18:2 n-6 *cis* LA	10.2 ± 0.0	25.5 ± 0.0
cis-9, *trans*-11 CLA	31.4 ± 0.0	0.1 ± 0.0
trans-10, *cis*-12 CLA	33.3 ± 0.1	0.3 ± 0.0
cis-10, *cis*-12 CLA	3.0 ± 0.0	nd
C20:4 n-6 AA	0.2 ± 0.0	0.3 ± 0.0
C24:1	0.1 ± 0.0	0.2 ± 0.0

Data are expressed as percentage share of a single fatty acid in the pool of all fatty acids. All data are shown as mean values ± standard deviation. AA—arachidonic acid; CLA—conjugated linoleic acid; LA—linoleic acid; OL—oleic acid.