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Cis-9. trans-11 conjugated linoleic acid against lipid peroxidation of rat brain homogenate and mitochondria

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ABSTRACT

In the present study it was evaluated the *in vitro* non-enzymatic lipid peroxidation of brain homogenate and mitochondria of two groups of Wistar rats: control and *c9, t11 conjugated linoleic acid* -supplemented. Lipid peroxidation was monitored by chemiluminescence and changes in the fatty acid composition by GLC. After incubation of brain homogenates and mitochondria in an ascorbate-Fe^{2+} system, it was observed that the total cpm originated from light emission, was lower, with no significant statistical differences, in those preparations isolated from CLA-supplemented group than in the control group The fatty acid composition was substantially modified during the lipid peroxidation with considerable decrease of polyunsaturated fatty acids, arachidonic acid C20:4! 6 and docosahexaenoic acid C22:6! 3 (PUFAs). In the control group the homogenate and mitochondria PUFAs contents diminished from 24.09 ± 1.35 to 10.76 ± 2.74 and 25.13 ± 4.34 to 12.25 ± 1.48%, respectively. No significant differences were observed when CLA group was compared to the control group. We conclude that in our experimental conditions, CLA did not protect the PUFAs from oxidative damage in the brain.

KEYWORDS

Chemiluminescence;
Polyunsaturated fatty acids;
Lipid peroxidation;
Conjugated linoleic acid;
Mitochondria;
Brain.

INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term generally referred to a mixture of positional and geometric conjugated dienoic isomers of linoleic acid[1]. Numerous CLA isomers are found in milkfat, cheese and beef[2, 3]. The cis-9, trans-11 isomer of CLA (the principal dietary form), is produced in the rumen of cattle and other ruminants during microbial biohydrogenation of linoleic and linolenic acids[4, 5]. CLA is an *in vitro* antioxidant, and the cells it protects membranes from oxidative attack[6]. The brain is vulnerable to oxidative damage due to its high content of polyunsaturated fatty acids (PUFAs) arachidonic acid (20: 4 n6) and docosahexaenoic acid (22: 6 n3) mainly, oxidative metabolism high and low antioxidant enzymes[7]. As lipoprotein structures enriched in polyunsaturated fatty acids are highly susceptible to attack by free radicals (lipid peroxidation). Mitochondria are the cellular compartment where oxidative metabolism occurs while where reactive oxygen species are generated therefore are most highly exposed to free radical damage organelles[8]. Oxidative stress has been linked to several neurological disorders[9]. Ali Y.M. *et*

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al. have demonstrated that CLA is a potent antioxidant and that cis-9, trans-11 isomer is selectively incorporated into cellular phospholipids, which may explain, at least in part, the anticarcinogenic activity of CLA as antioxidant[10]. The relationship between antioxidant-enzyme defense responses and cellular growth suppression in human cancer cells, exposed to CLA in cultures, has also been studied. The activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (Gpx) were induced in cell lines exposed to CLA. The data indicate that CLA-induced cytotoxicity against cancer lines is related to the extent of lipid peroxidation of CLA treated cells, and affirm that the CLA-induced antioxidant enzymes failed to protect these cells from cytotoxic lipid peroxidation products[11, 12]. One might speculate that the inhibition of carcinogenesis by CLA could result from the combined effects f a number of CLA activities, possibly including direct effects of one or more CLA isomers/metabolites on cell differentiation and the effects of one or more CLA isomers on prostaglandin metabolism, which may also influence cancer development at some sites[13, 14]. In previous works, was demonstrated that several membranes are protected by CLA, when subjected to non enzymatic lipid peroxidation[15, 16, 17]. The present study aims to compare the polyunsaturated fatty acid composition and non enzymatic lipid peroxidation of rat brain homogenate and mitochondria obtained from animals supplemented with conjugated linoleic acid.

MATERIAL AND METHODS

Female Wistar AH/HOK were obtained from Laboratory Animal Facility, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Butylated hydroxy-toluene (BHT) and phenylmethylsulfonyl fluoride (PMFS) were from Sigma Chemical Co. (St. Louis, MO, USA). BSA (Fraction V) was obtained from Wako Pure Chemical Industries Ltd, Japan. Standards of fatty acids methyl esters were kindly supplied by NU Check Prep. Inc, Elysian, MN, USA. L (+) ascorbic acid and boron trifluoride-methanol complex were from Merck Laboratories. All other reagents and chemicals were of analytical grade from Sigma.

Animals

Female Wistar AH/HOK rats 7 weeks old, weighing 120-137g were used. All rats were fed commercial rat chow and water *ad-libitum*. Two groups of 10 rats were considered, group A: received *c9, t11 conjugated linoleic acid* (CLA), group B: controls. Group A received daily 30 mg oral administration of CLA for 10 days. On day 11, all rats were sacrificed by cervical dislocation and the brains were rapidly removed.

Preparation of homogenate and mitochondria

The rats were sacrificed by cervical dislocation and the brain was rapidly removed, cut into small pieces and washed extensively with 0.15 M NaCl. An homogenate 30% (w/v) was prepared in a 0.25 M sucrose solution, 10 mM Tris-HCl pH 7.4 using a Potter-Elvejhem homogenizer. The homogenate was centrifuged at 3,000 G for 5 min; pellets were discarded and the supernatant was centrifuged at 10,000 G for 10 min to obtain mitochondrial pellets. The pellets were suspended in solution A (0.25 M sucrose solution, 10 mM Tris-HCl pH 7.4, 0.01 M PMFS). All operations were performed at 4 °C and under dim light.

Lipid peroxidation of rat brain homogenate and mitochondria

Chemiluminescence and lipid peroxidation were initiated by adding ascorbate to homogenate and mitochondria[18]. The homogenate and mitochondria from both groups, at a concentration of 0.5 mg of protein, were incubated at 37 °C with 0.01 M phosphate buffer pH 7.4, 0.4 mM ascorbate, final vol. 2 ml. Phosphate buffer is contamined with sufficient iron to provide the necessary ferrous or ferric iron (final concentration in the incubation mixture was 2.15 µM) for lipid peroxidation[19]. Homogenate and mitochondria preparations from both groups, which lacked ascorbate, were carried out simultaneously. Light emission was determined over a 180 min period, chemiluminescence was recorded as cpm every 10 min and the sum of the total chemiluminescence was used to calculated cpm/mg protein. Chemiluminescence was measured as counts per min in liquid scintillation analyzer Packard 1900 TR equipment with a program for chemiluminescence.

Fatty acid analysis

Homogenate and mitochondrial lipids were extracted with chloroform/methanol (2:1 v/v)[20] from na-

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tive or peroxidized membranes. Fatty acids were transmethylated with F_3B, in methanol at 60 °C for 3h. Fatty acids methyl esters were analyzed with a GC-14A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with packed column (1.80 m x 4 mm i.d.) GP 10% DEGS-PS on 80/100 Supelcoport. Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 250 °C; at the column temperature was held at 200 °C. Fatty acid methyl ester peaks were identified by comparison of the retention times with those of standard.

Protein determination

Proteins were determined by method of Lowry *et al.*[21] using BSA as standard.

Unsaturation index (UI)

UI= Sum % unsaturated acids x number of double bounds / Sum % saturated fatty acids

Statistical analysis

Results are expressed as means ± S.D. of six independent determinations. Data were evaluated statistically by one-way analysis of variance (ANOVA) and Tukey test. The statistical criterion for significance was selected at different p-values, which was indicated in each case.

RESULTS

The polyunsaturated fatty acid composition of total lipids isolated from mitochondria obtained from CLA and control groups is showed in TABLE 1.

The changes in fatty acids composition of organelles and unsaturation index from control group not show significant changes than those from CLA group. When the fatty acid composition and UI was analyzed between natives and peroxidized from each group significant differences were found the concentration of PUFAs (arachidonic acid C20:4ω6 and docosahexaenoic acid C22:6ω3) and UI. Similar results to those obtained in mitochondria, were found when was analyzed the fatty acid composition and UI of the homogenates obtained from group A and group B, TABLE 2.

The incubation of homogenates and mitochondria in the presence of ascorbate-Fe^{++} system resulted in the peroxidation of membranes, as evidenced by emission of light (chemiluminescence). It was observed that the cpm originated from light emission was not statistically significant when compared CLA treated group with control group. After incubation of brain homogenates and mitochondria in an ascorbate-Fe^{2+} system at 37°C during 180 min, it was observed that the total cpm originated from light emission chemiluminescence), was lower, no significant statistical differences, in those preparations isolated from CLA-supplemented group than in the control group, as an example homogenate: 1534,411 ± 80,391 and 1767,279 ± 39,064 cpm respectively. Thus, the chemiluminescence results agree

TABLE 1 : Fatty acid composition of brain mitochondria native and peroxidized obtained from rats control and supplemented with CLA

Fatty acid	Control group		CLA group	
	NATIVE	PEROXIDIZED	NATIVE	PEROXIDIZED
C16:0	21,08 ± 3,25	25,88 ± 2,99	22,04 ± 1,77	26,92 ± 2,86
C18:0	19,77 ± 1,75	19,74 ± 1,02	21,07 ± 0,57	21,10 ± 1,51
C18:1ω9	29,11 ± 2,13	36,36 ± 2,39	25,02 ± 0,72	39,72 ± 4,69
C18:2ω6	0,36 ± 0,81	0,44 ± 0,77	--------	0,57 ±0,98
C18:3ω3	2,25 ±1,69	2,32 ± 1,24	2,44 ± 0,33	0,80 ± 1,39
C20:4ω6	9,03 ± 1,25[a]	6,32 ± 0,07	9,17 ± 1,16[b]	4,53 ± 1,77
C22:6ω3	13,49 ±1,67[a]	3,16 ± 0,88	13,74 ± 1,78[b]	3,11 ± 0,67
Saturated	40,85 ± 3,57	45,62 ± 2,35	43,10 ± 2,04	48,02 ± 1,35
Monounsaturated	29,25 ± 2,00	36,36 ± 2,39	25,83 ±1,89	39,72 ± 4,69
Polyunsaturated	25,13 ± 4,34[a]	12,25 ± 1,48	25,35 ± 3,06[b]	9,01 ± 2,98
Total unsaturated	54,40 ± 2,45	48,61 ± 3,66	57,87 ± 8,49	48,73 ± 3,96
Saturated /unsaturated	0,75 ± 0,09	0,94 ± 0,12	0,75 ± 0,12	0,99 ± 0,07
Unsaturation Index	153,79 ±17,12[a]	88,44 ± 9,71	152,28 ± 17,28[b]	80,06 ±11,69

Data are given as the mean SD ± six experiments. [a]p <0.05 difference between native and peroxidized the control group. [b]p <0.05 difference between native and peroxidized the CLA group

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TABLE 2 : Fatty acid composition of brain homogenate native and peroxidized obtained from rats control and supplemented with CLA

Fatty acids	Control group		CLA group	
	NATIVE	PEROXIDIZED	NATIVE	PEROXIDIZED
16:0	21,94 ± 1.29	23,69 ± 0,39	22,84 ± 0,60	24,34 ± 0,28
18:0	19,95 ± 1.75	19,79 ± 0,98	18,37 ± 1,47	18,44 ± 1,91
18:1n-9	30,03 ± 2,93	37,81 ± 1,16	32,37 ± 1,53	37,19 ± 7,03
18:2n-6	0,49 ± 0,70	1,72 ± 1,15	-----	0,64 ± 0,91
18:3n-3	4,41 ± 0,59	1,33 ± 1,80	3,50 ± 1,42	2,78 ± 3,94
20:4n-6	7,76 ± 1,01[a]	4,38 ± 1,44	7,63 ± 1,63	4,23 ± 1,08
22:6n-3	11,43 ± 1,50[a]	3,33 ± 0,45	12,03 ± 1,44[b]	4,92 ± 0,67
Saturated	41,89 ± 2,99	43,47 ± 1,31	41,21 ± 1,84	42,78 ± 1,63
Monounsaturated	30,47 ± 2,84	37,81 ± 1,16	32,45 ± 1,42	37,19 ± 7,03
Polyunsaturated	24,09 ± 1,35[a]	10,76 ± 2,74	23,16 ± 3,49	12,57 ± 2,61
Total unsaturated	54,57 ± 2,37	48,57 ± 3,06	55,61 ± 2,13	49,76 ± 4,41
Saturated / unsaturated	0,77 ± 0,07	0,89 ± 0,03	0,74 ± 0,05	0,86 ± 0,04
Unsaturation Index	144,32 ± 7,41[a]	82,74 ± 11,30	145,61± 14,53 [b]	93,24 ± 2,63

Data are given as the mean SD ± six experiments. [a]p <0.05 difference between native and peroxidized the control group. [b]p <0.05 difference between native and peroxidized the CLA group.

with those obtained when was analyzed the fatty acid composition and UI.

DISCUSSION

The results of the present studies confirm previous communications of CLA incorporation in the brain. Conjugated linoleic acid (CLA) has been shown to exert several biological activities in different organs, in particular organs such as adipose and mammary tissue where CLA accumulates preferentially because of its high incorporation into neutral lipids[22, 23, 24]. However, despite numerous studies carried out in different experimental models, in vivo and in vitro, very little is known about the accumulation and metabolism of CLA in the brain. Dietary conjugated linoleic acid (CLA) causes reduced feed intake and body fat. It is unknown, though, if CLA incorporation into tissues, alterations in serum hormones, and/or appetite-regulating neuropeptides are involved[25, 26].

CLA incorporation into the brain has been detected only in few cases and at very low concentrations[27, 28]. The last finding could be ascribed to different reasons: (a) poor incorporation in phospholipids; (b) relatively low supply; (c) selective incorporation of other fatty acids; and (d) selection against unusual fatty acids with trans double bonds such as CLA. In contrast, there are no data available as to whether CLA metabolites are incorporated into the brain, as they are in several other tissues[29].

All research mentioned in this discussion are consistent with the results shown in this study when the composition of fatty acids (PUFAs), unsaturation index and lipid peroxidation (chemiluminescence) were analyzed.

Previous results show that CLA decrease polyunsaturated fatty acids[15, 16, 17]. Similarly, another author states that CLA does not appear to act as an antioxidant, its ability to decrease polyenoic fatty acid concentration could decrease of highly cytotoxic lipid peroxidation products[30]. As has been mentioned in this discussion, CLA incorporation into the brain has been detected only in few cases and at very low concentrations, this would explain because in our work we found no protection to supplement with CLA. For this reason we agree with the authors that suggest that CLA does not act as an antioxidant but decreasing polyenoic fatty acid concentration.

Future long-term feeding studies will be also needed, in order to establish whether incorporation of CLA and its metabolites in brain cells is able to modify the incorporation of arachidonic acid, as shown in other tissues[31]. Formation of eicosanoids could thus be interfered, either by decreasing their precursor or by incorporation of CLA metabolites shown to inhibit eicosanoid synthesis[32]. This could have a great

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impact on brain, since it is known that an increased inflammatory response may heavily contribute to the pathogenesis of neurological disorders such as Alzheimer syndrome, Parkinson disease and adrenoleukodystrophy[26]. Further studies are needed for a more adequate evaluation of these observations.

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REFERENCES

[1] S.Venkatramanan, S.V.Joseph, P.Y.Chouinard, H.Jacques, E.R.Farnworth, P.J.Jones; The journal of the american college of nutrition, **29**, 152-159 **(2010)**.
[2] S.V.Martins, P.A.Lopes, C.M.Alfaia, V.S.Ribeiro, T.V.Guerreiro, C.M.Fontes, M.F.Castro, G.Soveral, J.A.Prates; British Journal of Nutrition, **98**, 1206-1213 **(2007)**.
[3] L.M.Rodríguez-Alcalá, J.Fontecha; Journal of Dairy Science, **90**, 2083-2090 **(2007)**.
[4] L.Xu, Y.Jin, M.L.He, C.Li, K.A.Beauchemin, W.Z.Yang; Journal of Animal Science, **92**, 1089-1098 **(2014)**.
[5] C.Mapiye, J.L.Aalhus, T.D.Turner, D.C.Rolland, J.A.Basarab, V.S.Baron, T.A.McAllister, H.C.Block, S.D.Proctor, M.E.Dugan; Lipids, **49**, 275-286 **(2014)**.
[6] H.Stinshoff, S.Wilkening, A.Hanstedt, H.Bollwein, C.Wrenzycki; Reproduction Fertility and Development, **26**, 502-510 **(2014)**.
[7] S.H.Moussavi Nik, K.Croft, T.A.Mori, M.Lardelli; Zebrafish, **11**, 248-254 **(2014)**.
[8] G.Krumschnabel, M.Fontana-Ayoub, Z.Sumbalova, J.Heidler, K.Gauper, M.Fasching, E.Gnaiger; Methods in Molecular Biology, **1264**, 245-261 **(2015)**.
[9] E.Ginter, V.Simko, V.Panakova; Bratislavské lekárske listy, **115**, 603-606 **(2014)**.
[10] Y.M.Ali, A.A.Kadir, Z.Ahmad, H.Yaakub, Z.A.Zakaria, M.N.Abdullah; Pharmaceutical Biology, **50**, 712-719 **(2012)**.
[11] R.Devery, A.Miller, C.Stanton; Biochemical Society Transactions, **29**, 341-344 **(2001)**.
[12] S.Schønberg, H.E.Krokan; Anticancer Research, **15**, 1241-1246 **(1995)**.
[13] A.Heravi Moussavi, W.R.Butler, D.E.Bauman, R.O.Gilbert; american journal of veterinary research, **74**, 491-498 **(2013)**.
[14] A.P.Wakefield, M.R.Ogborn, N.Ibrahim, H.M.Aukema; The Journal of Nutritional Biochemistry, **23**, 908-914 **(2011)**.
[15] M.V.Scalerandi, M.A.Gonzalez, J.Saín, A.C.Fariña, C.A.Bernal; Nutrición Hospitalaria, **29**, 591-601 **(2014)**
[16] J.A.Fesler, D.G Peterson; Poultry Science, **92**, 2697-2704 **(2013)**.
[17] X.Qi, S.Wu, H.Zhang, H.Yue, S.Xu, F.Ji, G.Qi; Archives of Animal Nutrition, **65**, 354-365 **(2011)**.
[18] J.R.Wright, R.C.Rumbaugh, H.D.Colby, P.R.Miles; Archives of Biochemistry and Biophysics, **192**, 344–351 **(1979)**.
[19] B.Tadolini, G.Hakim; Free Radical Research, **25**, 221-227 **(1996)**.
[20] J.Folch, M.Lees, G.H.Sloane Stanley; The Journal of Biological Chemistry, **226**, 497-509 **(1957)**.
[21] O.H.Lowry, N.J.Rosebroughj, A.L.Farrl, R.J.Randall; The Journal of Biological Chemistry, **193**, 265-275 **(1951)**.
[22] C.Bothma, A.Hugo, G.Osthoff, C.C.Joubert, J.C.Swarts, H.L.De Kock; Meat Science, **97**, 277-286 **(2014)**.
[23] R.Kramer, S.Wolf, T.Petri; Lipids in Health and Disease, **4**, 12:96 **(2013)**.
[24] D.von Soosten, R.Kramer, G.Jahreis, U.Meyer, G.Flachowsky, S.Dänicke; Archives of animal nutrition, **67**, 119-133 **(2013)**.
[25] V.J.Shelton, A.G.Shelton, M.J.Azain, K.M.Hargrave-Barnes; Nutrition Research, **32**, 827-836 **(2012)**.
[26] M.Fa, A.Diana, G.Carta; Biochimica et Biophysica Acta, **1736**, 61-66 **(2005)**.
[27] L.Yang, Y.Huang, H.Q.Wang, Z.Y.Chen; Journal of Agricultural and Food Chemistry, **51**, 5654-5660 **(2003)**.
[28] C.Alasnier, O.Berdeaux, J.M.Chardigny, J.L.Sebedio; The Journal of Nutritional Biochemistry, **13**, 337– 345 **(2002)**.
[29] M.C.Mele, G.Cannelli, G.Carta; Prostaglandins, Leukotrienes and Essential Fatty Acids, **89**, 115-119 **(2013)**.
[30] S.Banni, A.Petroni, M.Blasevich; Lipids, **11**, 1143-1146 **(2004)**.
[31] D.Li, K.Yang, J.S.Li; International Journal of Nanomedicine, **7**, 6105-6114 **(2012)**.
[32] S.McClelland, C.Cox, R.O'Connor; Atherosclerosis, **211**, 96-102 **(2010)**.