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Conjugated Linoleic Acid Isomers and their Conjugated Derivatives Inhibit Growth of Human Cancer Cell Lines

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Abstract. *Conjugated linoleic acid (CLA), mainly c9,t11- and t10,c12-isomers, and polyunsaturated n-3 fatty acids (n-3 PUFA) have been shown to reduce tumor growth. This study compared, on a set of human tumor cells (breast, lung, colon, prostate and melanoma), the antiproliferative effects of: i) trans monounsaturated fatty acids (MUFA) vs. cis MUFA and MUFA vs. PUFA, ii) individual isomers of CLA vs. linoleic acid, iii) CLA-conjugated derivatives vs. their non-conjugated homologues and vs. CLA isomers. Tumor cells were exposed to medium containing individual FA (100 μM) for 48 h and their proliferation was determined by measuring the cellular DNA content (fluorescent Hoechst 33342 dye). The antiproliferative effects of FA varied with the type of cells and were mainly dependent on the degree of unsaturation and on the position and configuration of their double bonds. One isomer of CLA (t9,t11-18:2) and CLA-conjugated derivatives exhibited the strongest growth-inhibitory effect against cancer cells. These results suggest that ruminant products contain active compounds against human tumor cell proliferation.*

Human cancers partially result from various environmental factors (1) and, among them, dietary components could play an active role in the risk of development of various cancers, notably of the breast, colon, rectum and prostate (2). Indeed, fatty acid (FA) intake was proposed almost 60 years ago as a possible causal factor in the etiology of cancer (3). However, there are still many contradictions since, in experimental animals, cis monounsaturated and polyunsaturated FA of the (n-3) family ((n-3) PUFA) are protective against cancer development, whereas a diet high in (n-6) PUFA is correlated with cancer promotion (4). Moreover, several *in vitro* experiments using cell cultures all led to the same conclusion, that some FA, essentially (n-6) and (n-3) PUFA, when used at micromolar concentrations, possess cytotoxic or antiproliferative effects on tumor cells (5, 6). In the same way, conjugated linoleic acid (CLA), which is naturally present in ruminant products (meat, milk and dairy products), could have numerous potential benefits on human health, including anticarcinogenic properties (7-9). CLA is a collective term that refers to a mixture of positional and geometric isomers of linoleic acid with 2 conjugated double bonds in 4 geometric forms (*cis*/*trans, trans*/*cis, cis*/*cis* or *trans*/*trans*) located at various positions of the carbon chain. The presence of CLA in ruminant products results, on the one hand, from the bacterial biohydrogenation and *trans* isomerization of dietary PUFA in the rumen (10) and, on the other hand, from an endogenous synthesis in tissues by desaturation of vaccenic acid (VA, t11-18:1), another intermediate of the bacterial biohydrogenation of PUFA (11). These processes lead to the production of at least 24 isomers of CLA identified in dairy fats (12), being dominated by rumenic acid (c9,t11-CLA isomer, 80 to 90% of total CLA) (11). On the other hand, synthetic CLA isomers can be produced industrially by catalytic hydrogenation of vegetable oils, leading to the production of individual c9,t11- and t10,c12-isomers or, more often, in a mixture of both of these CLA isomers (usually in a 1:1 ratio), while the other positional and geometric isomers are present in much lower concentrations (13).

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CLA was first characterized as an anticarcinogenic factor from grilled beef on chemically-induced epidermal carcinogenesis in mice (14). This was the first of many *in vivo* but also *in vitro* studies on the anticarcinogenic properties of CLA. Indeed, dietary CLA supplementation (c9,t11 and/or t10,c12 isomers) inhibits the initiation and the development of mouse epidermal tumors (15), forestomach, cancer (16) and rat mammary cancer (17, 18). *In vitro*, the same isomers of CLA inhibit, in a dose- and time-dependent manner, the proliferation of human tumor cells from lung, breast, colon, skin, prostate and stomach cancers (19-21). Although the molecular mechanisms of the anticarcinogenic effects of CLA are still unknown, numerous studies showed that CLA inhibits DNA adduct formation induced by exposure to carcinogens, alters receptor-mediated actions of estrogens, activates the expression of genes implicated in transcription and signal transduction, induces caspase activation and apoptosis in tumor cells and inhibits angiogenesis *in vivo* (20, 22).

However, a majority of these *in vitro* and *in vivo* experiments were carried out using the 2 main isomers of synthetic CLA (c9,t11 and t10,c12-CLA isomers), tested either individually or in 50/50 mixture. Only one study reported that the antiproliferative properties of CLA were linked to the configuration of its conjugated double bonds (23). Indeed, among 3 CLA isomers tested (c9,t11-, t10,c12- and c9, c11-isomers), the *cis,trans* isomers exhibited a greater antiproliferative effect on both colo-rectal and prostate carcinoma cells than the *cis,cis* counterparts. In the same way, the antiproliferative properties of conjugated derivatives of CLA with 3 double bonds (C18:3 and C20:3) were never studied, although their synthesis by conversion of CLA has been previously described in the liver of rat (24-26), mice (27) and lamb (28).

In this context, the aim of the present study was to investigate, on a set of human tumor cells, the antiproliferative effects of: i) *trans* mono-unsaturated fatty acids (MUFA) compared to *cis* MUFA and MUFA compared to PUFA, ii) individual isomers of CLA (c9,t11; c9,c11; t9,t11; t10,c12; t7,c9 and c11,t13-isomers) compared to linoleic acid, iii) CLA-conjugated derivatives compared to their non-conjugated homologs (C18:3 and C20:3) and to CLA isomers.

Materials and Methods

Fatty acids. *Cis*-vaccenic (c11-18:1), vaccenic (t11-18:1), oleic (c9-18:1) and linoleic (c9,c12-18:2) acids were purchased from Sigma-Aldrich (L'Isle d'Abeau Chesnes, France). Linolenic acid (c9,c12,c15-18:3), *cis*-9,*trans*-11 (c9,t11)-CLA, *cis*-9,*cis*-11 (c9,c11)-CLA and *trans*-9,*trans*-11 (t9,t11)-CLA were purchased from Matreya Inc. (Pleasant Gap, PA, USA). Dihomo-gamma linolenic acid (c8,c11,c14-20:3) and *cis*-11,*cis*-14,*cis*-17 eicosatrienoic acid (c11,c14,c17-20:3) were purchased from Nu-Chek Prep Inc. (Elysian, MN, USA). *Trans*-10,*cis*-12 (t10,c12)-CLA, *trans*-7,*cis*-9 (t7,c9)-CLA, *cis*-11,*trans*-13 (c11,t13)-CLA, *cis*-6,*trans*-10,cis-12 (c6,t10,c12)-18:3, *cis*-8,*trans*-12,*cis*-14 (c8,t12,c14)-20:3 and *cis*-8,*cis*-11,*trans*-13 (c8,c11,t13)-20:3 were synthesized in our laboratory as previously described (29). Stock solutions of each fatty acid in ethanol (100 mM) were kept at –20˚C until use.

Cell lines and culture conditions. M4Beu, a human melanoma cell line, was established in the laboratory of Dr. J.F. Doré (INSERM, Unit 128, Lyon, France) from metastatic biopsy specimens and has been maintained in cell culture for almost 15 years (30). Breast adenocarcinoma (MCF7) and carcinoma (T47D), prostatic adenocarcinoma (PC3), colon adenocarcinoma (DLD-1) and lung non-small cell carcinoma (A-549) human cell lines were purchased from the European Collection of Cell Cultures (ECACC, Salisbury, UK). This set of human tumor cells was chosen because of the high impact of such cancers on the human death rate (31). Stock cell cultures were maintained as monolayers in 75-cm^2 culture flasks in complete medium containing: Glutamax Eagle's Minimum Essential Medium (MEM) with Earle's salts (Gibco-BRL, Paisley, UK, ref. 41090-28) supplemented with 10% fetal calf serum naturally poor in CLA (Biochrom, batch 431 B, France) as verified by gas liquid chromatography analysis (data not shown), 1% vitamin solution (Gibco-BRL, ref. 11102-037), 1% sodium pyruvate solution (Gibco-BRL, ref.11360-039), 1% mixture of non-essential amino-acids (Gibco-BRL, ref. 11140-035) and 2 mg gentamicin base (Gibco-BRL, ref. 15710-049). All cell culture solutions were certified endotoxin-tested and sterile-filtered. The cells were grown at 37˚C in a humidified incubator and under an atmosphere containing 5% CO_2 during a 2-week period of adaptation before the proliferation assay. The same batch of fetal bovine serum was systematically used for all experiments to minimise effects due to inter-batch variability.

Proliferation assay. Preliminary experiments were performed to determine the appropriate concentration and duration of FA supplementation: dose-effect curves (from 10 to 500 μM) showed a toxic effect of FA from 200 μM (data not shown) and time-course analysis performed for 72 h showed similar results between 48 and 72 h (data not shown). Consequently, the cells were plated at density of $5x10^3$ per 150 μL culture medium in 96-well microplates (Nunclon, Nunc, Roskild, Denmark) and allowed to adhere for 16 h before treatment with FA. The medium was then replaced by a fresh complete culture medium supplemented with a given individual FA (3 wells by treatment) at the final FA concentration of 100 μM in a the final volume of 200 μL. In these conditions, the final concentration of ethanol in the culture medium was 0.25% for all experiments. In parallel, control cells were incubated in ethanol (0.25% vol/vol.). The correct proliferation of control cells was assessed daily by light microscopy observations and was statistically similar to that of cells grown with oleic acid, indicating the biological neutrality of our control (23). After 48 h of continuous FA exposure, the antiproliferative effect of FA was assessed by measurement of the DNA content with Hoechst dye 33342 (32). Briefly, 100 μL of SDS solution (0.01%, m/v) was distributed into each well, the plates were incubated for 1 h at room temperature and frozen at –80˚C for 1 h. After thawing, 100 μL of Hoechst dye 33342 solution at 30 μg/mL in a hypersaline buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 2 M NaCl) was added to each well. The plates were incubated in this solution for 1 h, protected from light at room temperature on a plate shaker. Fluorescence was measured at 360/460 nm on a microplate fluorescent reader. Three independent experiments were performed, each in triplicate.

Statistical analysis. The values are expressed as the mean±SEM for 3 independent experiments. The global effects of cell type and FA nature were tested by analysis of variance (ANOVA) using the GLM procedure in SAS (33). The effects tested in the model included the nature of the FA tested, the type of cell line and the interaction between the type of cells and the nature of the FA tested. Significance was set at $p<0.05$. In addition, the means of each family of FA were compared by orthogonal contrast statements (33). In this context, the whole of the MUFA was compared with linoleic and linolenic acids, the whole of the CLA isomers was compared with linoleic acid, both c9,t11- and t10,c12-CLA isomers were compared with their 18:3 and 20:3 conjugated derivatives, and the whole of conjugated 18:3 and 20:3 FA was compared with their non-conjugated homologs.

Results

Global growth inhibitory activity of fatty acids on human tumor cell lines. Global statistical analysis of the means of all the experiments, expressed as a percent of the control, showed a growth-inhibitory activity of all FA ($p=0.0001$), which varied according to the responses of the cancer cell lines ($p=0.0001$) without any interaction between the cell lines and FA tested ($p=0.461$). To simplify the results, the FA were grouped as great families, and comparisons of their growth inhibitory activities were performed using orthogonal contrast statements.

Intensity of response of each tumor cell line to fatty acids. The intensities of response of the tumor cell lines to FA were different (Tables I to IV). Indeed, the lung (A-549), colon (DLD-1) and breast (T47D and MCF7) tumor cell lines were relatively sensitive, whereas the prostatic (PC3) and melanoma (M4Beu) tumor cell lines were more resistant to FA treatment.

Effects of fatty acids on proliferation of human tumor cells. a) Monoenoic and non-conjugated polyenoic acids. Among the 3 C18 monoenoic acids tested, the configuration of the double bond did not modify the growth inhibitory activity of these FA, except on the A-549 and DLD-1 cell lines (Table I). However, the values were too close to those of the control to reveal a biological significance. Among the *cis* C18 polyenoic acids, the decrease of cell proliferation by linolenic acid (c9,c12,c15-18:3) was always higher than that of linoleic acid (c9,c12-18:2), this difference being significant in the MCF-7 and A-549 cell lines (+31.8 and +49.3% of activity, respectively, $p<0.01$) and in the T47D line (+21.5% of activity, $p<0.05$). Monoenoic acids were less effective than the polyenoic acids (significant orthogonal contrasts), in inhibiting the growth of all the human tumor cell lines mainly because of the high antiproliferative activity of linolenic acid. Consequently, the growth-inhibitory activity of a FA was significantly and positively correlated to the number of double bonds of the FA ($r=0.88$, $p<0.05$, data not shown).

b) CLA isomers. All CLA isomers tested significantly decreased tumor cell proliferation in the MCF-7 and T47D cell lines (Table II). Orthogonal contrasts indicated no significant difference of growth-inhibitory activity between linoleic acid and CLA isomers, except for a tendency in the MCF-7 cell line ($p<0.087$). However, among the CLA isomers, the t9,t11-CLA isomer was significantly more active than linoleic acid in the T47D and A-549 cell lines (+34.6 and +53.8% of activity, respectively, $p<0.05$). Moreover, this isomer was significantly more efficient in inhibiting cell proliferation than the t10,c12- and c9,t11-isomers in the MCF-7 line (+34.8 and +33.1% of activity, respectively, $p<0.05$), than the c9,t11-, t7,c9- and c9,c11- isomers in the T47D line (+25.8, +33.2 and +30.6% of activity, respectively, $p<0.05$) and than the t7,c9- and c11,t13-isomers in the A-549 line (+45.6 and +52.6% of activity, respectively, $p<0.05$).

c) Conjugated CLA derivatives. Products of the desaturation and elongation of c9,t11-CLA (c8,c11,t13-20:3) and of t10,c12-CLA (c6,t10,c12-18:3 and c8,t12,c14-20:3), as well as non-conjugated 18:3 and 20:3 FA, significantly decreased tumor cell proliferation from 24.5 to 83.6 % when compared to the control (Table III). The orthogonal contrast analysis showed that the proliferation of the human tumor cell lines was similar when the medium was supplemented with the 18:3 and 20:3 conjugated derivatives of CLA or with non-conjugated 18:3 and 20:3 FA, except in the MCF-7 line, where CLA-conjugated derivatives were less efficient than their non-conjugated homologs ($p<0.05$), mainly because of the low inhibitory activity of the c8,c11,t13-20:3 isomer.

d) CLA isomers and derivatives. The c9,t11- and t10,c12-CLA isomers were compared with their derivatives, c8,c11,t13-20:3 produced by the bioconversion of c9,t11-CLA, and c6,t10,c12-18:3 and c8,t12,c14-20:3 produced by the bioconversion of the t10,c12-CLA isomer (Table IV). Global analysis by orthogonal contrast showed that the CLA derivatives did not possess a different growth-inhibitory activity on human tumor cell proliferation than their precursors. However, when analyzed individually, c6,t10,c12-18:3 and c8,t12,c14-20:3 were more efficient in inhibiting cell proliferation than the CLA isomers in MCF-7 and M4Beu lines (around +27.7 and +26.4% of activity, respectively, $p<0.05$), whereas, in PC3, c6,t10,c12-18:3 and c8,c11,t13-20:3 were more effective than the CLA isomers (+37.2 and +35.6% of activity, respectively, $p<0.05$).

Discussion

The role of dietary polyunsaturated FA in the regulation of tumor cell growth presents a conundrum in cancer research. FA of the same chain length can have different effects on tumor cell growth depending on the number, position and configuration of their double bonds (1, 4). Among dietary

Table I. *Effects of the configuration and the number of double bonds present in non-conjugated FA on human tumor cell proliferation.*

	trans MUFA	*cis* MUFA		*cis* PUFA			Effect of FA	Orthogonal contrasts	
	t11-18:1	c9-18:1	c11-18:1	c9,c12-18:2	c9,c12,c15-18:3	SEM	($p=$)	*trans vs. cis* MUFA	MUFA *vs.* PUFA
	number of cells (% control)								
MCF7	71.0◆[a]	78.5◆[a]	83.7[a]	69.4◆[a]	47.3◆[b]	3.86	0.0040	NS	0.0017
T47D	79.5◆[de]	80.4[de]	91.3[d]	76.8◆[e]	60.3◆[f]	5.23	0.0143	NS	0.0060
A-549	94.7a	105.6[a]	119.3[b]	98.1[a]	49.7◆[c]	8.09	0.0003	0.014	0.0003
DLD-1	78.1	90.4	100.7	81.6	70.7	6.29	0.0240	0.072	0.0531
M4beu	95.7d	105.3[d]	102.3[d]	88.6[de]	72.4◆[e]	2.91	0.0360	NS	0.0069
PC3	101.6	111.6	90.6	82.7	77.2	1.04	NS	NS	0.0951

Values are means of 3 independent experiments±SEM.
Black rhombus (◆) indicates a significant difference compared to control ($p<0.05$).
Significant differences between FA ([a,b,c] : $p<0.01$, [d,e,f] : $p<0.05$).

Table II. *Effects of CLA isomers compared to their non-conjugated homolog on human tumor cell proliferation.*

	non-conjugated FA		CLA isomers					Effect of FA	Orthogonal contrast
	c9,c12-18:2	t10,c12-18:2	c9,t11-18:2	t7,c9-18:2	c9,c11-18:2	c11,t13-18:2	t9,t11-18:2	SEM	($p=$)	non-conjugated *vs.* conjugated FA
	number of cells (% control)									
MCF7	69.4◆[de]	81.6◆[d]	79.5◆[d]	68.4◆[de]	64.1◆[de]	61.9◆[de]	53.2◆[e]	1.48	0.106	0.087
T47D	76.8◆[d]	59.6◆[ef]	67.7◆[df]	75.2◆[d]	72.4◆[df]	52.0◆[e]	50.2◆[e]	1.27	0.007	NS
A-549	98.1[d]	68.3[de]	71.3[de]	83.3[d]	68.4[de]	95.6[d]	45.3◆[e]	2.65	0.034	NS
DLD-1	81.6	78.3	69.8	74.0	66.0	84.2	62.0◆	5.36	NS	NS
M4beu	88.6	102.3	84.6	87.5	100.7	78.0	85.4	1.16	NS	NS
PC3	82.7	107.0	95.5	95.3	103.5	80.6	73.3	0.61	NS	NS

Values are means of 3 independent experiments ± SEM.
Black rhombus (◆) indicates a significant difference compared to control ($p<0.05$).
Significant differences between FA ([d,e,f] : $p<0.05$).

PUFA, CLA is believed to have many beneficial properties for humans including anticarcinogenic effects (7). The majority of the *in vitro* and *in vivo* studies investigating the anticancer effects of CLA have been conducted either with individual or with a 50/50 mixture of the 2 main CLA isomers (c9,t11- and t10,c12-18:2) produced industrially from partially hydrogenated vegetable oils (34). In contrast, the present study investigated, for the first time, the antiproliferative effects of biological isomers of CLA found in ruminant products, such as minor CLA isomers with *cis,cis* or *trans,trans* configuration, and of conjugated CLA derivatives resulting from CLA elongation and desaturation reactions.

In our experiment, fetal calf serum (10%) was added to the cell culture medium to mimic physiological conditions and to take into account the possible competition between CLA and the other FA present in the serum for their uptake by cells and their subsequent intracellular metabolism, as occurs *in vivo*. However, under these experimental conditions, the antiproliferative effects of CLA (approximately 70% of control cells) were lower than those observed in the majority of *in vitro* experiments performed without serum (up to 100% of inhibition of proliferation at the same range of concentration) (35). These effects could be explained by the simultaneous effects of CLA treatment and serum deprivation. Serum deprivation in itself may cause the sensitivity of the cells to CLA to increase responses to CLA. The levels of FA used in the present study (100 μM) were similar to those used in different human cancer cell cultures,

Table III. *Effects of CLA-conjugated derivatives compared with their non-conjugated homologs on human tumor cell proliferation.*

	non-conjugated homologs		CLA-conjugated derivatives				Effect of FA	Orthogonal contrast
	c9,c12,c15-18:3	c8,c11,c14-20:3	c6,t10,c12-18:3	c8,c11,t13-20:3	c8,t12,c14-20:3	SEM	($p=$)	non conjugated *vs.* conjugated FA
	number of cells (% control)							
MCF7	47.3◆^{d}	48.2◆^{d}	56.4◆^{de}	75.5◆^{e}	60.0◆^{de}	2.04	0.08	0.022
T47D	60.4◆	60.3◆	61.7◆	69.9◆	69.6◆	0.46	NS	NS
A-549	49.7◆	31.3◆	58.7◆	34.6◆	53.7◆	0.98	NS	NS
DLD-1	70.7	57.8◆	61.1◆	60.7◆	64.5	1.25	NS	NS
M4beu	72.4◆	56.1◆	69.3◆	79.5	67.9◆	1.21	NS	NS
PC3	77.2	76.2	63.6◆	65.2◆	78.7	0.99	NS	NS

Values are means of 3 independent experiments ± SEM.
Black rhombus (◆) indicates a significant difference compared to control ($p<0.05$).
Significant differences between FA (^{d,e} : $p<0.05$).

Table IV. *Effects of CLA isomers compared to their CLA-conjugated derivatives on human tumor cell proliferation.*

	CLA		CLA-conjugated derivatives				Effects of FA	Orthogonal contrast
	t10,c12-18:2	c9,t11-18:2	c6,t10,c12-18:3	c8,c11,t13-20:3	c8,t12,c14-20:3	SEM	($p=$)	CLA *vs.* derivatives
	number of cells (% control)							
MCF7	81.6◆^{d}	79.5◆^{d}	56.4◆^{e}	75.5◆^{de}	60.0◆^{e}	2.45	0.074	NS
T47D	59.6◆	67.4◆	61.7◆	69.9◆	69.6◆	0.50	NS	NS
A-549	68.3	71.3	58.7◆	34.6◆	53.7◆	4.74	NS	NS
DLD-1	78.3	69.8	61.1◆	60.7◆	64.5◆	6.14	NS	NS
M4beu	101.8^{d}	84.6^{e}	69.3◆^{f}	79.5◆^{ef}	67.9◆^{f}	9.71	0.005	NS
PC3	107.0^{d}	95.5^{d}	63.6◆^{e}	65.2◆^{e}	78.7^{de}	2.57	0.039	NS

Values are means of 3 independent experiments ± SEM.
Black rhombus (◆) indicates a significant difference compared to control ($p<0.05$).
Significant differences between FA (^{d,e,f} : $p<0.05$).

such as colorectal (23), prostatic (36) and breast cancer cells (37). Physiological concentrations of CLA in human sera were in the 10-70 μM range. However, levels up to 5 times higher than those found in normal sera have been achieved in humans following long-term supplementation with CLA (37). Therefore, the CLA concentration of 100 μM used in the present study corresponded to physiological levels.

The intensity of response of the 6 human cancer cell lines to any tested FA each differed fom the others as previously demonstrated in several experiments (23, 38, 39). These differences of cellular sensitivity could be explained by intrinsic differences in cell lines, such as the different histogenic origin of tumor cells, the variable rate of cell proliferation, the selective cellular uptake of FA and their cellular use.

Effects of the number of double bonds. The significant positive correlation between the degree of unsaturation of double bonds and the growth-inhibitory activity of FA demonstrated in the present cell experiment was in agreement with previous data (6, 40). In the same way, conjugated derivatives of CLA which contain 3 double bonds exhibited a greater activity of inhibition of cell proliferation than CLA isomers. Because of the high level of PUFA in polar lipids of cell membranes (41), lipid peroxidation is thought to be one of major mechanisms explaining the inhibition of tumor cell growth by PUFA (42). Indeed, membrane PUFA could favor the generation of free radicals and lipid peroxides (43, 44), which could damage the DNA and, thus, inhibit cell proliferation (42). Moreover, several studies have shown that a higher level of

lipoperoxidation is positively correlated to a lower cell growth in several types of cancer (5, 45). Thereby, the sensitivity of FA to peroxidation, which depends on the number of double bonds (44), could explain, at least in part, the relationships observed in the present study between the growth-inhibitory activities of FA and their degree of unsaturation.

Effects of the configuration of double bonds. For the same degree of unsaturation, FA sensitivity to peroxidation would be directly linked to the number of allylic centers in their structure (46). Thus, a 18-C FA which contains 2 conjugated double bonds and then 2 sites of hydrogen abstraction could be less sensitive to peroxidation than its non-conjugated conterpart which possesses 4 allylic centers. However, under our experimental conditions, CLA and their conjugated derivatives exhibited the same antiproliferative activity against cancer cells as their non-conjugated homologs. This suggested the existence of additional mechanisms of action of CLA and of their derivatives, such as a decrease in arachidonate-derived eicosanoid synthesis, this latter being implied in several health disorders, notably in cancer (47). Further studies are thus necessary to clarify with precision how these properties of CLA, and probably of their conjugated derivatives, might contribute to decrease the proliferation of cancer cell lines.

Effects of the configuration of double bonds. In addition to the degree of unsaturation and to the position and the conjugation of double bonds, the effect of the double bond configuration was studied by comparing the growth-inhibitory activities of 9,11-CLA isomers which have *cis,trans, cis,cis* or *trans,trans* configurations. Among the 6 CLA isomers tested, the t9,t11- CLA isomer appeared to be the most potent inhibitor of the growth of the cancer cells. A recent study, which compared the growth-inhibitory activities of c9,t11-, t10,c12- and c9,c11-CLA isomers on human colorectal and prostate cancer cells, suggested that *cis,trans* conjugated bonds could have a greater inhibitory effect against cancer cell proliferation than their *cis,cis* counterparts (23). These differences between CLA isomers could be explained either by differences in CLA isomer uptake by cells and/or by specific mechanisms of action of these CLA isomers (48), since it has been shown, on prostate cancer cells, that t10,c12-CLA acts preferentially through the modulation of apoptosis and of cell cycle control, whereas the c9,t11-isomer affects arachidonic acid metabolism (36). Further studies are therefore necessary in order to clarify the specific pathway of action of each class of CLA isomers.

In conclusion, the comparison of the antiproliferative effects of unsaturated FA with 1, 2 or 3 double bonds, as well as those of CLA isomers and of their conjugated derivatives, demonstrated that the growth-inhibitory activity of these FA against human cancer cell lines depended on the degree of unsaturation and on the position and the configuration of their double bonds. Moreover, this work showed that one isomer of CLA (*i.e.* t9,t11-18:2) and CLA-conjugated derivatives possessed important growth-inhibitory effect against cancer cells. As these compounds are present in ruminant products (meat and milk), it is of interest to investigate the antiproliferative effects of CLA mixtures from ruminant fats and their mechanisms of action, since possible relationships between the FA (synergy and/or antagonism) might influence the anticarcinogenic properties of such natural mixtures.

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