Mammary Cancer Prevention by Conjugated Dienoic Derivative of Linoleic Acid

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ABSTRACT

Conjugated dienoic derivative of linoleic acid (CLA) is a collective term which refers to a mixture of positional and geometric isomers of linoleic acid. It is a naturally occurring substance in food and is present at higher concentrations in products from animal sources. The present study reports that synthetically prepared CLA is an effective agent in inhibiting the development of mammary tumors induced by dimethylbenz(a)anthracene. Rats were fed either the AIN-76A basal diet or the same diet supplemented with 0.5, 1, or 1.5% CLA by weight. These diets were started 2 weeks before carcinogenesis and continued until the end of the experiment. The total number of mammary adenocarcinomas in the 0.5, 1, and 1.5% CLA groups was reduced by 32, 56, and 60%, respectively. The final tumor incidence and cumulative tumor weight were similarly diminished in rats fed the CLA-containing diets. In general, there appeared to be a dose-dependent protection at levels of 1% CLA and below, but no further beneficial effect was evident at levels above 1%. Chronic feeding of up to 1.5% CLA produced no adverse consequences in the animals. Analysis of the phospholipid fraction from liver and mammary tumor extracts showed that only the c9,t11 isomer of CLA was incorporated and that the level of incorporation increased with dietary intake. An interesting property of CLA is its ability to suppress peroxide formation from unsaturated fatty acid in a test-tube model. In view of this information, the amount of thiobarbituric acid-reactive substances (lipid peroxidation products) present endogenously in liver and mammary gland was quantitated. The feeding of CLA (for either 1 or 6 months) resulted in a decrease in the extent of lipid peroxidation in the mammary gland, but such a suppressive effect was not detected in the liver. It should be noted that maximal antioxidant activity was observed with only 0.25% CLA in the diet, whereas maximal tumor inhibition was achieved at about 1% CLA. Hence there is a discrepancy between the antioxidant efficacy of CLA and its anticarcinogenic potency, suggesting that some other mechanisms might be involved in cancer protection. Unlike the stimulatory effect of linoleic acid in carcinogenesis, the reaction of CLA in cancer prevention is specific, and CLA is more powerful than any other fatty acid in modulating tumor development.

INTRODUCTION

Conjugated dienoic derivative of linoleic acid is a collective term which refers to a mixture of positional and geometric isomers of linoleic acid (c9,c12-octadecadienoic acid). The two double bonds in CLA are primarily in positions 9 and 11, or 10 and 12, along the carbon chain, thus giving rise to the designation of a conjugated diene. Each of the double bonds could be in the cis or trans configuration. Theoretically, eight possible geometric isomers of 9,11- and 10,12-octadecadienoic acid (c9,c11; c9,t11; c9,c12; c9,t12; c10,c12; c10,t12; t10,c12; t10,t12) would form from the isomerization of c9,c12-octadecadienoic acid. CLA is a naturally occurring substance in food. It was initially isolated and identified as an anticarcinogenic agent from grilled ground beef and then shown to be present in a variety of dairy products as well. The initial data from Pariza's laboratory indicated that the collection of isomers, c9,t11-, r10,c12- r9,t11-, and r10,t12-octadecadienoic acids are the four major derivatives that account for more than 90% of total CLA, while the c9,c11-, r9,c11-, c10,c12-, and c10,t12-octadecadienoic acids represent only minor constituents. Recently, it was further shown that meat from ruminants in general contains more CLA than meat from nonruminants. For example, the former group is estimated to contain 3 to 6 mg CLA/g of fat, while the latter has (with one exception) less than 1 mg CLA/g of fat.

Previous work has examined synthetically prepared CLA (containing the four major isomers) for antinitiation activity in the two-stage mouse epidermal carcinogenesis model (1). CLA was topically applied at 7 days (at a dose of 20 mg/mouse), 3 days (20 mg), and 5 min (10 mg) before DMBA treatment. Control mice were instead painted with linoleic acid prior to DMBA. All mice were given 12-O-tetradecanoylphorbol-13-acetate for tumor promotion. It was found that CLA reduced the number of papillomas by one-half compared to those in the linoleic acid-treated controls. More recently, the synthetic CLA mixture has also been shown to inhibit benzo(a)pyrene-induced forestomach tumors in mice (4). In this experiment, a dose of 0.1 ml CLA was administered by gavage at 4 and 2 days prior to treatment with benzo(a)pyrene during the first week, and this sequence was repeated for 4 consecutive weeks.

We report in the present study the feeding of CLA on the development of mammary cancer induced by DMBA in rats. Synthetically prepared CLA, in the form of free fatty acids, was added directly to the basal AIN-76A diet. In our protocol, three levels of CLA feeding (0.5, 1, and 1.5%) were started 2 weeks before DMBA administration and continued until the end of the experiment. The rationale for adopting this design was based on the previous observation that CLA could inhibit the metabolism of DMBA in an in vitro rat liver microsomal system. We fully realized that this design would not allow us to differentiate the effect of CLA on the initiation versus the promotion stage of carcinogenesis. On the other hand, we had no a priori knowledge of whether CLA would be effective in cancer prevention under a chronic feeding schedule. On this basis, we decided to expose the animals to CLA before, during, and after DMBA treatment in order to maximize the probability of a positive outcome.

It should be pointed out that the levels of CLA used in this study are much higher than would normally be attained through the consumption by humans of CLA-containing foods in the diet. A 300-g rat fed a 0.5% CLA diet will consume about 0.075 g of CLA/day. In a direct extrapolation to a 70-kg person, this is equivalent to a daily CLA intake of about 17 g, a figure considerably higher than the estimated consumption of 1 g/person/day in the United States. However, because of certain constraints in the experimental design (high dose of carcinogen, short duration of exposure before carcinogenic insult,
and limited sample size), it is necessary to use a generous quantity of CLA in these feeding studies. The incorporation of CLA in livers and mammary tumors was examined in order to determine the correlation with the level of intake. Other biochemical studies were also carried out in which the effects of CLA on tissue lipid peroxidation and phase II detoxifying enzymes were investigated.

MATERIALS AND METHODS

Animals, Diet, and Mammary Tumor Induction. Pathogen-free female Sprague-Dawley rats at 30 days of age were purchased from Charles River Breeding Laboratories (Raleigh, NC) and housed in polycarbonate cages in a room with a 12-h light/12-h dark cycle. They were acclimatized immediately to the powdered AIN-76A diet (substituting dextrose for sucrose) as described previously (5). One week later, the colony was randomized to 5 different groups (30 rats/group) and given their CLA-containing diets: Group 1, basal diet with no CLA; Group 2, 0.5% CLA (by weight); Group 3, 1.0% CLA; Groups 4 and 5, 1.5% CLA. CLA was added at the expense of carbohydrate and mixed with the other ingredients of the diet. Water and food were available ad libitum throughout the experiment. The AIN-76A diet contains 5% corn oil, which has been estimated to consist of approximately 0.2 mg CLA/g of fat (3). Thus it can be calculated that the basal AIN-76A diet contains about 1 mg of CLA/100 g, an amount that is negligible compared to the quantities of CLA added to the diets of Groups 2 to 5.

Two weeks after the start of the CLA-supplemented diets, rats in Groups 1 to 4 were given an oral intubation of 10 mg of DMBA (Sigma) dissolved in 1 ml of corn oil for induction of mammary tumors. Those in Group 5 received the corn oil vehicle only and therefore served as the negative control. The body weights of rats in Groups 1 to 5 at the time of DMBA treatment were (mean ± SE) 183 ± 2, 180 ± 2, 181 ± 2, 181 ± 2, and 180 ± 2 g, respectively. All animals were palpated weekly to determine the appearance and location of tumors. The experiment was terminated 24 weeks after DMBA administration; the CLA-containing diets were fed continuously during the entire period. At autopsy, the mammary gland was exposed for the detection of nonpalpable tumors. All tumors were excised for histological examination. Only confirmed adenocarcinomas were reported in the results (6). Tumor incidences at the final time point were compared by x2 analysis, and the total tumor yield was compared by frequency distribution analysis as described previously (7).

Analysis of CLA Incorporation in Tissues. Liver, mammary gland, and mammary tumor samples from Groups 1 to 4 of the above carcinogenesis experiment were obtained at autopsy and analyzed for incorporation of CLA into the phospholipid fraction. Details of the methodology have been described in a previous publication (4). Total fat was extracted by chloroform:methanol (8), and phospholipids were separated by silicic acid column chromatography (9). Fatty acid methyl esters were then prepared and extracted with hexane (10). Total CLA methyl esters were eluted from reversed-phase high-performance liquid chromatography, and individual CLA isomers were quantitated by capillary GC (2).

Biochemical Studies. A short-term feeding experiment was carried out in which rats were fed diets containing 0.25, 0.5, 1.0, or 1.5% CLA for 1 month. In addition, two other groups were included that were given diets containing either 0.05% vitamin E (all-rac-a-tocopherol acetate) or 0.1% BHA. Control rats were fed the basal AIN-76A diet. None of the animals involved in this study received DMBA. The extent of endogenous lipid peroxidation was evaluated in liver and mammary gland homogenates by the method of Okhawa et al. (11). This method quantitates the amount of thiobarbituric acid-reactive substances (presumably lipid peroxidation products) present in tissues, using malondialdehyde as the standard. Lipid peroxidation was similarly examined in the liver and mammary gland samples obtained from the DMBA carcinogenesis experiment at the time it was terminated.

Two phase II detoxifying enzymes, glutathione-S-transferase and UDP-glucuronyl transferase, were also studied. The activity of glutathione-S-transferase in the postmitochondrial supernatant (24,000 × g for 1 h) was measured by the method of Habig et al. (12) with 1-chloro-2,4-dinitrobenzene as the substrate. The activity of UDP-glucuronyl transferase in the 105,000 × g microsomal pellet was measured by the method of Lucier et al. (13) using p-nitrophenol as the acceptor. Both assays were performed only in liver and mammary gland samples from the 1-month CLA feeding experiment in which the animals were not treated with DMBA.

Preparation of CLA and Purity Determination. CLA was prepared in batches as free fatty acids at the Kraft General Foods Technology Center (Glenview, IL). Five hundred g of linoleic acid (99+% pure; Aldrich Chemical) were added to a 5-liter, 3-neck flask containing 150 g of sodium hydroxide (AR grade) dissolved in 2900 g of ethylene glycol. The apparatus was equipped with a mechanical stirrer, a thermometer, a reflux condenser, and a nitrogen inlet. The mixture was heated at 180°C under an inert atmosphere for 2 h. The reaction mixture was cooled to ambient conditions, and 320 ml of concentrated HCl were added. After 15 min of stirring, the pH was adjusted to 4 with additional HCl. The reaction mixture was then transferred to a 4-liter separatory funnel and extracted with two 500-ml portions of hexane. The combined hexane solution was extracted with three 250-ml portions of 5% NaCl, dried over 3-Å molecular sieves, filtered through a sintered glass Buchner funnel, and then placed in a rotary evaporator. After the removal of hexane, the remaining CLA was cooled with ice prior to breaking the vacuum. The purified CLA was placed in plastic bottles, capped in a nitrogen atmosphere, and stored frozen at −20°C.

GC analysis of the CLA methyl ester derivatives, extracted with hexane, was carried out with a Hewlett Packard 5890 model fitted with a flame ionization detector. In order to preserve the natural isomeric distribution, derivatization was performed by the direct addition of boron trifluoride/methanol to the saponified CLA. The column used was a J & W Scientific DB-wax capillary column (30 m x 0.32 mm inner diameter, 0.25-μm film thickness). GC conditions were: injector temperature, 200°C; split ratio, 80–100:1; carrier gas, helium at 12 psi; column temperature, 100°C for 2 min, then to 180°C at 20°C/min, then to 230°C at 2°C/min, with a 15-min hold; detector temperature, 250°C. The volume injected was 1 μl. Purity was calculated by normalization of the GC profile of the methyl esters, under the assumption that the peaks had a similar response to the flame ionization detector. The percentage of each isomer was then calculated from the total peak area.

A total of 13 batches of CLA were prepared for feeding in the mammary cancer prevention experiment and biochemical studies. The results of the GC analysis of all these batches showed the following composition (mean ± SE): e9,11- and e9,c11-octadecadienoate, 42.5 ± 0.7%; r10,c12-octadecadienoate, 43.0 ± 0.7%; e9,c11-octadecadienoate, 1.3 ± 0.3%; c10,c12-octadecadienoate, 1.2 ± 0.3%; r9,t11- and r10,t12-octadecadienoate, 2.3 ± 0.3%; e9,c12-octadecadienoate (unchanged parent compound), 6.5 ± 1.1%; and remainder (unidentified), 3.3 ± 0.4%. Thus, three particular isomers, i.e., e9,t11-, e9,c11, and r10,c12-octadecadienoates, accounted for 85% of the total isomers in the CLA preparation that was fed to the animals.

RESULTS

Mammary Cancer Suppression by CLA. Fig. 1 illustrates the cumulative appearance of mammary adenocarcinomas in Groups 1 to 4 as a function of time after DMBA administration. Animals in Group 5, which were fed the 1.5% CLA diet but were not treated with DMBA, did not develop any palpable mammary adenocarcinomas and were therefore not represented in this figure. In those rats that were given a dose of carcinogen at about 50 days of age, the feeding of CLA produced an appreciable reduction in the cumulative appearance of mammary adenocarcinomas and were therefore not represented in this figure (6). Tumor incidences at the final time point were compared by x2 analysis, and the total tumor yield was compared by frequency distribution analysis as described previously (7).

Two phase II detoxifying enzymes, glutathione-S-transferase and...
However, a graded dose response between 0.5 and 1.0% CLA became apparent in the second half of the study. Increasing the dietary level of CLA to 1.5% did not appear to offer further protection against neoplastic development of the mammary gland compared to 1.0% CLA under the present experimental conditions.

Table 1 summarizes the data on tumor incidence, total tumor yield (including nonpalpable tumors discovered at autopsy), tumor multiplicity, and total tumor weight from each group. Only the 1.0 and 1.5% CLA diets produced a statistically significant reduction in tumor incidence. All three CLA-containing diets resulted in a decrease in the total number of mammary adenocarcinomas compared to that found in the control group \( (P < 0.05) \). The magnitude of inhibition ranged from 32% in the 0.5% CLA group to 60% in the 1.5% CLA group. In general, the changes in total tumor weight in each of the four DMBAs-treated groups paralleled that of tumor yield. The number of fibroadenomas found in Groups 1 to 5 was on the order of 10, 7, 5, 4, and 1, respectively. Thus CLA inhibits the development not only of malignant tumors but benign tumors as well.

Chronic CLA feeding had no effect at all on the growth rate of the animals. As a matter of fact, the body weight curves of all 5 groups of rats were so close to each other that it would be impossible to plot them together in a single graph and still be able to distinguish the individual lines. Consequently, the body weights were presented at selected time points in Table 2 to support the observation that CLA feeding did not influence the growth of the animal. Periodic measurements of food intake (once a month for 5 days at a time) showed that there was no difference among the five groups (data not presented). At four different times during the course of the experiment, vaginal smears were taken from 6 rats in each group in order to follow estrus cycle regularity. There was no noteworthy deviation observed among the CLA-fed rats. Table 2 also shows the organ weights at autopsy. The weights of liver, spleen, kidney, and uterus from the CLA groups were all comparable to those obtained from the control group. Nothing unusual was detected upon visual inspection of these organs from all 150 rats. Histological sections of three randomly chosen samples of each organ from Groups 1 and 4 revealed no morphological abnormality in tissues taken from rats fed the 1.5% CLA diet (data not shown). One of the authors has carried out an independent study in which 1.5% CLA was fed to male rats for 36 weeks. At the end of the experiment, a pathologist evaluated the histology of 15 different tissues from these animals and found no evidence of histomorphological abnormality. Thus, it can be concluded that chronic feeding of CLA at the levels tested here has no apparent adverse effect on either male or female animals.

Incorporation of CLA in Phospholipid. CLA incorporation in the phospholipid fraction of liver and mammary tumor samples was investigated and reported in Table 3. The rationale for studying CLA incorporation into mammary tumors was to compare the utilization of CLA in a malignant tissue and in a normal nontarget tissue such as the liver. Although rats were fed a CLA preparation which contained a mixture of isomers (refer to “Materials and Methods”), only the c9, t11 isomer was found to be incorporated into phospholipids. Results in Table 3 show that even in control rats which did not receive any CLA supplementation in the diet, a low but measurable level of CLA in phospholipids was detected in both liver and mammary tumors. There was a progressive elevation in CLA incorporation with increasing intake of dietary CLA. In the liver, maximal incorporation was reached between 1.0 and 1.5% CLA so that there was about a 6-fold increase compared to the control. No evidence of saturation was observed in the mammary tumors in the range between 0.5 and 1.5% dietary CLA. Furthermore, the magnitude of increase of CLA incorporation was greater in the mammary tumor in comparison to the liver. A diet containing 1.5% CLA resulted in a 20-fold increase of CLA incorporation in the mammary tumor over the control value.

We intentionally did not report the incorporation of CLA in the mammary gland. The reason is because of the confounding effect due to tissue heterogeneity, which makes it difficult to interpret the data. Mammary gland from virgin rat consists primarily of adipocytes and stromal tissue. Fat in adipocytes is stored in the form of triglycerides. Previous work has shown that all isomers of CLA appear in triglycerides, whereas only the c9, t11 isomer is found in phospholipids. The phospholipid fraction of the whole mammary gland constitutes only about 1% of the total amount of extractable fat, whereas the corresponding values for liver and mammary tumor are around 40 and 20%, respectively. Thus it is technically difficult to analyze for the small amount of specific incorporation of the c9, t11 isomer in the phospholipid fraction of the mammary gland. Despite this reservation, we did try to quantitate the level of CLA in the mammary gland phospholipids and found that it increased with dietary CLA intake. However, because of potential contamination by triglycerides which are present in much larger proportion in the mammary tissue, the validity and significance of this observation remained somewhat unclear.

Antioxidant Activity of CLA. Previous work has shown that CLA is a potent antioxidant (4). At a molar ratio of 1 part CLA to 1000 parts linoleic acid in a test-tube model, peroxide formation from linoleic acid was inhibited by more than 90%. In view of this information, we decided to quantitate the amount of thiobarbituric acid-reactive substances present endogenously in liver and mammary gland homogenates obtained from rats that had been fed diets containing different levels of CLA for 1 month. As noted in “Materials and Methods,” these animals...
were not treated with DMBA. This study also included two additional groups which were supplemented with either 0.05% vitamin E or 0.1% BHA. Both vitamin E and BHA were effective antioxidants and could therefore serve as positive controls for comparing the activity of CLA. Results in Table 4 show that dietary CLA had no effect on the amount of thiobarbituric acid-reactive substances in the liver, but a significant decrease was detected in the mammary gland following CLA feeding. Interestingly, there was no dose response in the decrease of thiobarbituric acid-reactive substances in the liver, but a significant decrease was detected in the mammary gland following CLA feeding. ALA-mediated inhibition of lipid peroxidation compared to the liver was again found to be less sensitive to inhibition by CLA, vitamin E, or BHA. Thus other methods for assessing lipid peroxidation should also be used in the future in order to confirm the results reported here.

On account of the above observation, the effect of chronic feeding of CLA on the formation of thiobarbituric acid-reactive substances was also examined. Tissue samples were randomly selected from Groups 1 to 4 of the DMBA carcinogenesis experiment. Thus the animals used here had been fed the CLA-containing diets for more than 6 months. Results of the assay are shown in Table 5. In the mammary gland, dietary CLA levels ranging from 0.5 to 1.5% produced the expected decrease in thiobarbituric acid-reactive substances, thus confirming the data from the short-term feeding study. Lipid peroxidation in the liver was again found to be less sensitive to inhibition by CLA. Under the long-term feeding condition, only the highest level of CLA at 1.5% led to a slight, although significant,
CLA Feeding and Phase II Detoxifying Enzymes. Antioxidants such as BHA are known to induce glutathione transferase activity (14). The ability of CLA to cause similar changes in this enzyme in the liver and mammary gland was investigated using samples from the 1-month feeding study. As shown in Table 6, unlike vitamin E and BHA, CLA produced no stimulation in the activity of glutathione transferase. Since CLA was preferentially incorporated in tissue phospholipid fraction with increased intake (Table 3) and might therefore modulate microsomal enzyme activity, a microsomal phase II detoxifying enzyme, i.e., UDP-glucuronol transferase, was measured in these same samples from rats given 0.25 to 1.5% CLA. There was no significant change in the liver UDP-glucuronol transferase activity of rats following CLA feeding (data not shown). The activity of UDP-glucuronol transferase was so low in the mammary gland with our assay system that it was not feasible to draw any valid conclusion regarding the effect of CLA on this enzyme in this particular organ.

DISCUSSION

This is the first report in the literature which shows that CLA feeding via the dietary route is effective in cancer prevention. Of the naturally occurring substances that have been demonstrated to have anticarcinogenic activity in experimental models, all but a handful of them are of plant origin (15-18). CLA is therefore unique since it is present in higher concentration in food from animal sources (2,3). It should be pointed out that CLA is not exclusively a constituent of animal products. Plant oils, such as peanut, corn, or safflower oils, also contain CLA but at a much lower level when expressed per unit weight of fat (3). Fish oil is a class of lipid which has been reported by many investigators to inhibit both chemical-induced and transplantable tumors (19-22). CLA provides a good example that fats from ruminant (beef, lamb, and dairy products) and nonruminant (pork, chicken, and turkey) sources also contain some component that has powerful cancer protective activity.

Perhaps it would be informative to put in perspective the potency of CLA relative to other fatty acids which have been known to modulate tumorigenesis. CLA is closely related to linoleic acid but differs from linoleic acid in the position and configuration of the double bonds. But unlike the stimulatory effect of linoleic acid on carcinogenesis, CLA inhibits tumor development (1,4). Using the DMBA-induced mammary cancer model, Ip et al. (23) have previously shown that the yield of tumors progresses in proportion to increasing intake of linoleic acid from 0.5 to 4% by weight in the diet. The specific require-

Table 6  Effect of CLA, vitamin E, or BHA on glutathione-S-transferase activity in liver and mammary gland*

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<th>Glutathione-S-transferase activity*</th>
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<td>0.05% vitamin E</td>
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<td>0.1% BHA</td>
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* CLA, vitamin E, or BHA was added to the basal AIN-76A diet and fed to the animals for 1 month.

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Ha et al. (4) found that in mice that were given a preparation of CLA containing a mixture of isomers, only the c9,t11-CLA isomer was incorporated into forestomach phospholipids, suggesting that this may be the biologically active isomer. Similar findings with the liver and mammary tumor confirmed the characteristic attribute of the c9,t11 isomer (Table 3). What is the physiological significance of CLA incorporation into phospholipids in relation to inhibition of tumorigenesis? It seems paradoxical that CLA would protect against malignancy but is itself incorporated in increasing amounts in the tumors with increasing dietary intake. Changes in CLA incorporation are not achieved at the expense of linoleic acid (4), suggesting that there is a certain degree of specificity involved in the process. It is possible that CLA may interfere with some early events in cell transformation, so that in those initiated colonies which have escaped the CLA-mediated suppressive mechanism(s), the proliferating neoplastic cells will continue to incorporate CLA since they are no longer sensitive to the biological effects of CLA.

So far, CLA has been found to inhibit tumorigenesis in three different models: DMBA/12-O-tetradecanoylphorbol-13-ace-
tate two-stage induction of skin papillomas (1), benzo(a)pyrene-induced forestomach tumors (4), and DMBA-induced mammary tumors. The mechanism by which CLA acts as an anticarcinogen is not clear. In the cultured mouse fibroblast system, CLA has been found to shift benzo(a)pyrene metabolism away from activation and toward detoxification. Since much of the activation of benzo(a)pyrene in fibroblasts is mediated by peroxidative mechanism rather than by cytochrome P-450, these results provide additional support for the thesis that CLA acts as an antioxidant in vivo as well as in vitro. Based on the observation that CLA could inhibit the metabolism of DMBA in an in vitro rat liver microsomal system (1), we intentionally started the CLA-containing diets prior to the administration of DMBA in the mammary cancer prevention study. Although we failed to detect any changes in the phase II detoxifying enzymes subsequent to CLA feeding, potential interference of DMBA activation cannot be ruled out. Further studies on DMBA metabolism need to be correlated with the efficacy of CLA feeding only during the initiation phase of DMBA-induced mammary carcinogenesis. The protective activity of CLA in the postinitiation phase would also have to be validated since the design of the current study precluded a direct answer regarding this mode of action of CLA. The fact that the c9,11 isomer is preferentially incorporated into membrane phospholipid fraction would suggest a possible locus of action along the signal transduction pathway that might impact on neoplastic proliferation. This should clearly be a major emphasis of future research.

An interesting property of CLA is its ability to suppress peroxide formation from unsaturated fatty acids that are exposed to air and heated at an elevated temperature for a prolonged period of time (4). In fact, CLA is superior to α-tocopherol in this regard. There is nothing in the structure of CLA per se to suggest that it should possess such activity. It is hypothesized that an oxidized derivative of CLA is the active antioxidant species rather than CLA itself (4). According to a currently proposed scheme which is supported by in vitro and in vivo UV spectrophotometric evidence, a β-hydroxy acrolein moiety is introduced across the conjugated double-bond system following reaction with a hydroxyl or peroxyl radical and molecular oxygen (4). Antioxidant activity most likely results from chelation of iron by the β-hydroxy acrolein functional group, thereby interfering with the Fenton reaction (26). Both synthetic and naturally occurring antioxidants with diverse structures have been known to inhibit malignancy (27). Could the anticarcinogenic activity of CLA be attributed solely to its antioxidant propensity? The answer is equivocal with the limited information available. Using the thiobarbituric acid assay as an index of lipid peroxidation, we have found that dietary CLA is an effective antioxidant, but only in the mammary tissue. We have no explanation as to why peroxidation in the liver is much less sensitive to inhibition by CLA. Second, maximal antioxidant activity was observed with 0.25% CLA in diets (Table 4), whereas maximal tumor suppression was achieved at about 1% CLA. Thus there is a discrepancy between antioxidant efficacy and anticarcinogenic activity, suggesting that some other mechanism(s) might be involved.

Current research on the biology and biochemistry of CLA is still in its infancy. Recent analytical data collected under a better controlled condition indicate that about 90% of total CLA in dairy products is in the form of the c9,11-isomer. This is consistent with the observation that rumen microorganisms preferentially isomerize c9,c12-octadecadienoic acid to c9,t11-octadecadienoic acid (28). In contrast, the synthetically prepared CLA that we used for the feeding study contained eight different isomers, with the c9,t11 isomer accounting for no more than 42% of the total. If the c9,t11 isomer is the biologically active species, since it is the only form incorporated in membrane phospholipids, the possibility remains that a preparation which is enriched in the c9,t11 isomer may have an even higher anticarcinogenic activity. The development of new technology in synthesizing CLA that is abundant in the c9,t11 isomer should facilitate future research in evaluating the efficacy and potential of CLA in cancer prevention.

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