SYMPOSIUM: A BOLD NEW LOOK AT MILK FAT

Conjugated Linoleic Acid and Other Anticarcinogenic Agents of Bovine Milk Fat

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ABSTRACT

Prevention is an important strategy for conquering cancer. Milk fat contains a number of components, such as conjugated linoleic acid, sphingomyelin, butyric acid, ether lipids, β-carotene, and vitamins A and D that have anticancer potential. Conjugated linoleic acid inhibits the growth of a number of human cancer cell lines and suppresses chemically-induced tumor development at a number of sites in animal models. As little as 0.1% of dietary conjugated linoleic acid inhibits the development of rat mammary tumors, independent of the amount and type of fat in the diet. Sphingomyelin, through its metabolites ceramide and sphingosine, participates in multiple antiproliferative pathways associated with suppression of carcinogenesis. Dietary sphingomyelin inhibits murine colon tumor development. Butyric acid, uniquely present in ruminant milk, is a potent antineoplastic agent and may ameliorate its potency through synergy with other milk fat components. Dietary butyric acid inhibits mammary carcinoma development in rats. In humans, ether lipids, β-carotene, and vitamins A and D are associated with anticancer effects. Cows have the ability to extract anticarcinogenic components from pasture and feed and transfer them to milk. Use of genetic engineering and other techniques to increase the range and level of anticarcinogens in pasture and supplements may increase the anticancer potential of milk.
(**Key words**: milk fat, anticarcinogens, conjugated linoleic acid, butyric acid)

Abbreviation key: **ACF** = aberrant crypt foci, **CLA** = conjugated linoleic acid, **DMBA** = 7-12-dimethylbenz[a]anthracene, **HMG CoA** = 3-hydroxy-3-methylglutaryl coenzyme A, **LDL** = low density lipoprotein, **MNU** = methylnitrosourea, **IQ** = 2-amino-3-methylimidazo[4,5-*f*]quinoline.

INTRODUCTION

Despite the immense expenditure worldwide on cancer research during the past 30 yr, and the notable advances made in this field, the death rate for patients with invasive and metastatic carcinoma of the colon, breast, lung, pancreas, prostate, and bladder have not decreased very much (84). Thus prevention, rather than therapy, must be an important strategy for conquering cancer. Although cancer will never be completely eradicated, as long as genes continue to mutate spontaneously, the hazards of environmental carcinogens from smoking, industrial pollutants, ultraviolet radiation, and diet can be largely eliminated.

Diet is thought to account for about one-third of all cancer deaths in affluent populations with a range of 20 to 60% for the various sites (15). Our diets contain components that may either help cause cancer or help prevent cancer (2). There is increasing awareness that a high consumption of fruit and vegetables is associated with a lowered risk of cancer at most sites, due to their content of numerous anticarcinogenic agents (2). The future, no doubt, will have not only greater reliance on vegetable-based diets but also the introduction of genetically engineered foods containing components that block the initiation and progression of malignancy. This presents a challenge for animal science to produce products that can compete in this crucial field.

This review identifies and discusses the action of a number of anticarcinogenic components in bovine milk fat, specifically, conjugated linoleic acid (**CLA**), sphingomyelin, butyric acid, ether lipids, β-carotene, and vitamins A and D. Further, the review canvasses the possibility of genetically engineered pasture and feed to introduce other types of anticarcinogens that may subsequently be transferred to milk.

CLA

The acronym CLA refers to various positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid) for which the two double bonds have a conjugated arrangement instead of methylene

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interruption. The biological properties of dietary CLA are currently attracting considerable interest because of its diverse physiological outcomes in animal studies. Not only is CLA a powerful anticarcinogen, but it also has antiatherogenic, immunomodulating, growth promoting, and lean body mass-enhancing properties (54). Antidiabetic properties for CLA, which are striking, have also been reported recently (32).

Anticarcinogenic Properties

Interest in CLA as an anticarcinogen grew from the observation by Pariza and his colleagues that both raw and grilled ground beef contained a component that could inhibit mutagenesis. This inhibitor was later shown to possess anticarcinogenic properties (54). The anticarcinogen was purified and identified as four isomers of linoleic acid with conjugated diene unsaturation (25). These isomers (CLA) were synthesized by base-catalyzed isomerization of linoleic acid and used subsequently in a number of studies that showed the isomers suppressed tumor development in a range of animal models and inhibited growth in many cancer cell lines (61).

Animal studies. Mouse epidermal tumors, initiated by 7, 12-dimethylbenz[a]anthracene (**DMBA**) and promoted by 12-*O*-tetradecanoylphorbol-13-acetate, have been suppressed by topical application of CLA (25). Similar tumor reduction was obtained with this model when CLA was provided as a 1.0 or 1.5% dietary supplement (4). Mouse forestomach tumors induced with benzo[a]pyrene were inhibited by CLA administered by gavage (26).

The heterocyclic amine 2-amino-3-methylimidazo [4,5-*f*] quinoline (**IQ**), a common product of food pyrolysis, is a potent mutagen and carcinogen. Mice fed CLA prior to IQ administration had reduced IQ-DNA adducts in a number of organs (98). Rats fed CLA were protected against IQ-adduct formation in the colon (45). Administration by gavage of CLA, equivalent to a dietary intake of 0.5%, inhibited the number of IQ-induced colonic aberrant crypt foci (**ACF**) (45), which are microscopically determined preneoplastic lesions and are considered the earliest precursors of colon cancer. When rats were exposed to the dietary mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, the addition of 0.1 to 1.0% CLA to their diet inhibited mutagen-DNA adduct formation in the colon and mammary gland (78).

Cell culture studies. Physiological concentrations of CLA inhibited the growth of human malignant melanoma and colorectal and breast (MCF-7) cancer cell lines (80). In another study, CLA exerted a dose-dependent reduction in proliferation of three human lung adenocarcinoma cell lines but had no effect on glioblastoma cells. In contrast, linoleic acid had no inhibitory effect on the cell lines (77). Melanoma, leukemia, mesothelioma, and glioblastoma together with breast, prostate, colon, and ovarian cancer cell lines (91) and two human hepatoma cell lines (95) had their growth inhibited by CLA. A primary canine prostate cell line, but not a prostate cell line that produced lung metastasis, was inhibited by CLA (9).

CLA and Prevention of Breast Cancer

A number of studies, reviewed elsewhere (21, 70), show that linoleic acid enhances the development of mammary tumors in rodents. In contrast, a growing number of studies show that its isomer, CLA, inhibits tumor development, which offers an exciting prospect for dietary prevention of breast cancer.

Cell culture studies. Shultz et al. (79) reported that human MCF-7 breast cancer cell growth was inhibited by CLA in a dose- and time-dependent manner. Linoleic acid did not produce comparable results. Later, they found that linoleic acid stimulated the growth of normal human mammary epithelial cells, whereas CLA was inhibitory (10). Visonneau et al. (91) also found that CLA inhibited the growth of five different breast cancer cell lines and confirmed that linoleic acid tended to stimulate cell growth. Durgam and Fernandes (16) found that, although CLA inhibited the growth of MCF-7 cells that are estrogen receptor positive, CLA did not inhibit the growth of MDA-MB-231 cells that are estrogen receptor negative. This result indicates that CLA inhibited growth by interfering with a mitogenic pathway that was regulated by hormones.

Animal studies. Dietary studies with rat mammary tumor models have established CLA as a potent anticarcinogen. In a seminal study, Ip et al. (37) commenced feeding CLA to 37-d-old rats, 2 wk before administration of the carcinogen DMBA. Supplementation of a basal diet with 0.5, 1.0, or 1.5% CLA resulted in a reduction in tumor incidence (percentage of rats with tumors) of 17, 42, and 50%, respectively. The total number of mammary adenocarcinomas was reduced by 32, 56, and 60%, respectively. A follow-up study (41) showed that when the dose of DMBA was halved and tumors took longer to occur, dietary CLA at concentrations between 0.05 to 0.5% produced a dose-dependent inhibition of tumor incidence and tumor yield (total tumors per treatment group). Subsequent studies by Ip and colleagues

found, that the anticancer action of CLA was similar when fed as either the inexpensive and readily available free fatty acid or as the prohibitively expensive but natural triglyceride form (40). Next, the magnitude of inhibition of DMBA-induced mammary tumors by a diet supplemented with 1% CLA was not influenced by the concentration (10, 13.3, 16.7, or 20%) of fat in the diet. Furthermore, tumor inhibition by 1% CLA was equally effective when fed as part of a 20% unsaturated fat diet, represented by corn oil, or a 20% saturated fat diet, represented by lard (36).

Rats were fed diets containing either 2 or 12% linoleic acid, adjusted to a total of 20% fat with coconut oil. These diets were supplemented with 0.0, 0.5, 1.0, 1.5, and 2.0% CLA. As expected, mammary tumor incidence induced by DMBA was higher in the 12% linoleic acid fed groups at all concentrations of supplementation than in groups fed 2% linoleic acid. However, the percentage of tumor inhibition at each concentration of CLA supplementation was similar for both the 2 and 12% linoleic acid diets. By increase of the CLA concentration from 0.5% to 1.0%, both diets produced a dose-dependent decrease in tumor incidence and total tumor number. No further protection was apparent at the higher concentrations of CLA. Thus, the efficacy of tumor suppression by CLA is not affected by linoleic acid intake (39).

Using a different model, Visonneau et al. (92) fed severe combined immunodeficient mice diets that were supplemented with 1% CLA 2 wk before subcutaneous injection of human breast adenocarcinoma (MDA-MB468) cells. Dietary CLA inhibited local tumor growth by 73% at 9 wk. Also, CLA prevented metastatic spread to the lungs, peripheral blood, and bone marrow. On the other hand, Wong et al. (93) found that supplementation with up to 0.9% CLA did not prevent the growth of metastatic murine mammary tumor cells infused into the right inguinal mammary gland of Balb/c mice.

Of immense significance are the observations of Ip and his colleagues that the age of the rat, at the time when CLA feeding commences, is crucial to mammary tumor development outcome. Initially, Ip et al. (41) fed rats from weaning at 21 d of age a diet supplemented with 1% CLA. This diet was continued for 5 wk, 1 wk after carcinogen administration; the animals then consumed the control diet until the end of the experiment 36 wk later. Two carcinogens were used, DMBA and methylnitrosourea (**MNU**). Compared with the control diet, 1% CLA supplementation reduced mammary tumor incidence in DMBA-treated rats from 80 to 52% and in MNU-treated rats from 88 to 60%. In a follow-up experiment, rats were fed 1% CLA from weaning (21 d) and were injected with MNU at 42 d or 56 d. In both cases, CLA was removed from the diet after MNU treatment. Tumor incidence was reduced by 32% and 39%, respectively, for CLA-fed rats receiving MNU at 42 d and 56 d of age. In addition, the total number of tumors was reduced by 47 and 42%, respectively (40). Next rats were fed 1% CLA immediately after MNU administration at 56 d of age for 1, 2, or 5 mo. Short-term exposure to CLA for 1 or 2 mo was ineffective in mammary tumor protection. Significant protection was obtained only in the rats that received an uninterrupted supply of CLA (40). A similar result was obtained when DMBA was used as the carcinogen (38). Recently, Thompson et al. (86) treated rats with DMBA at 50 d of age when they were divided into four treatment groups. The groups received either a control diet or a diet supplemented with 1% CLA commencing (1) from weaning to 50 d of age, (2) from 55 d to the end of the experiment at 21 wk post-DMBA treatment, or (3) from weaning to the end of the experiment. Compared with the control group, percentage of inhibition of mammary tumors for the three treatments was 49, 54, and 57% respectively.

Morphology and Pathogenesis

The above studies by Ip and his colleagues (38, 40, 41, 86) are notable because, first, they illustrate that dietary CLA is effective in suppressing mammary tumor development during the prepromotion, promotion, and progression phases of carcinogenesis. The second notable aspect is lifelong protection from mammary cancer when CLA is fed only during a short period of about 3 wk from weaning and before chemically-induced tumor promotion. However, if CLA feeding commences later in life and after tumor promotion is initiated, then, lifetime supplementation is required to obtain equivalent protection.

Mammary carcinogenesis is dependent on the age and physiological development of the mammary gland at the time of tumor promotion. Albanes and Winick (1) point out that cancer risk is proportional to the number of proliferating cells, which, in turn, depends on both the number of cells and the rate of cell division within the tissue. Further, tumor incidence increases when carcinogen exposure takes place during periods of rapid cell division. Shorter cell cycles may lessen the degree of DNA repair occurring prior to the next division, thereby permitting greater inheritance of genomic errors.

For the rat, the interval from weaning at 21 d of age until about d 50 represents a period of active morphogenesis during which the mammary gland ma-

tures to adult stage morphology. The mammary gland evolves from a primary lactiferous duct, arising from the nipple, which branches into multiple secondary ducts. With increasing age, the number and length of these ducts increase while the ends form club-shaped terminal end buds. The number of terminal end buds is highest at 21 d of age, and they comprise 3 to 6 layers of actively proliferating epithelial cells. After d 21, the terminal end buds begin to cleave into three to five smaller differentiated alveolar buds. About this time, some terminal end buds become smaller with an atrophic appearance. These are called terminal ducts. Differentiation of terminal end buds into alveolar buds is accentuated by each estrous cycle that starts when the animals are 35 to 42 d of age (72).

Mammary tumorigenesis that is chemically induced occurs primarily in the rapidly proliferating epithelium at the distal end of terminal end buds. Differentiation of cells is associated with not only altered gland structure but also with lengthening of the cell cycle, mainly the G1 phase; decreased binding of the carcinogen to DNA; and increased ability of the cells to remove carcinogen-adducts from DNA (72).

The observations on carcinogenesis in the developing rat mammary gland have correspondence in human studies. There is growing opinion that the early stages of breast cancer begin at a young age (11) and even as early as the intrauterine period of development (50). Incidence of breast cancer among atomic bomb survivors at Hiroshima and Nagasaki was several-fold higher for women who were under 10 yr of age at exposure (87). Epidemiological and clinical observations indicate that breast cancer incidence is greater in nulliparous women, whereas early parity confers protection. Russo et al. (71) reported that parous women with breast cancer had less differentiated gland structure (resembling nulliparous women) than did parous women without cancer.

Mechanisms. Given the above observations, does the anticarcinogenic action of CLA stem from its ability to inhibit proliferation and promote differentiation in mammary epithelial cells? Mechanisms for CLA action, although often studied, are still largely unresolved. Various studies have suggested that CLA may act by antioxidant mechanisms (26, 37), prooxidant cytotoxicity (77), inhibition of nucleotide and protein synthesis (80), reduction of cell proliferative activity (10, 41), and inhibition of both DNA-adduct formation (98) and carcinogen activation (25, 45).

However, Thompson et al. (86) have now shown, by digitized image analysis, that treating rats with 1% CLA from weaning until about 50 d of age caused a 21% reduction in the density of the epithelium from the ductal-lobular tree. This result was accompanied by a 30% suppression of epithelial proliferative activity in the terminal end buds and lobuloalveolar buds. Thompson et al. (86) also found that feeding 1% CLA increased by 65-fold the content of CLA in mammary tissue. In addition, there was a 6- and 14-fold increase in $C_{18:3}$ and $C_{20:3}$ fatty acids with conjugated unsaturation, respectively. This illustrates that CLA underwent both desaturation and elongation in vivo.

Holman et al. (31) claim that unusual isomers of polyunsaturated fatty acids can inhibit the metabolism of normal polyunsaturated acids, such as linoleic acid, at many steps in the normal metabolic cascade. They can also be precursors of unusual eicosanoids or inhibit the synthesis of normal eicosanoids. The most important eicosanoid precursor is arachidonic acid ($C_{20:4}$), which is synthesized from linoleic acid (a promoter of mammary tumors) by elongation and desaturation. Arachidonic acid is stored in cell membranes esterified to the *sn*-2 position of phospholipids. Eicosanoids derived from the arachidonic acid cascade have been implicated in mammary tumor development, possibly by interaction with growth factors and oncogenes. Mammary tumors can be inhibited by agents that interfere with the arachidonic acid cascade (70). The anticarcinogenic action of CLA may, in part, be explained by its ability to inhibit arachidonic acid-derived eicosanoids.

Milk Fat CLA

In ruminant animals, CLA is produced as a stable first intermediate in the biohydrogenation of dietary linoleic acid by a linoleic acid isomerase from the rumen bacteria *Butyrivibrio fibrisolvens* (44). In the second step of the pathway, the conjugated diene is hydrogenated to *trans*-11-octadecenoic acid. Further hydrogenation results in stearic acid. Parodi (57) established by chemical reductionism, using a combination of reductive ozonolysis and hydrazine reduction, that milk fat CLA was almost entirely *cis-9*, *trans*-11-octadecadienoic acid, which is now referred to as rumenic acid.

All studies demonstrating anticarcinogenic action of CLA used a synthetic mixture. Ha et al. (26) and Ip et al. (37) found that all isomers were incorporated into tissue triglycerides. However, only the *cis*-9, *trans*-11-isomer was incorporated into membrane phospholipids and is considered to be the biologically active isomer. The synthetic CLA contains only about 40% of this isomer. Milk fat CLA is almost entirely *cis*-9-*trans*-11-$C_{18:2}$ (57), which should be noted when transposing the results of animal studies to a likely human situation.

Milk fat is the richest natural source of CLA with reported values ranging from 2.4 to 28.1 mg/g (69). Seasonal variation is very marked with values during the summer period often up to 2 or 3 times higher than winter values. Fat from meat and organs of ruminant animals also contains appreciable amounts of CLA (8, 23). Reported CLA values for Australian and New Zealand milk and meat fat are often some 2 to 3 times higher than values reported for equivalent United States products. This phenomenon presumably reflects the greater access to lush pasture, rich in polyunsaturated fatty acids, throughout the year by Australasian cattle and sheep (60, 61).

Because of the potential health benefits arising from CLA consumption, there is considerable research effort directed to increasing the CLA content of ruminant-derived food. Salient findings are those of Kelly et al. (43) who reported that feeding a high linoleic acid oil (sunflower) increased CLA concentrations to 24.4 mg/g of milk fat compared with values of 13.3 and 16.7 mg/g of fat for high oleic and high linolenic acid oils, respectively. Dhiman et al. (12) found that cows grazing on pasture could attain a CLA concentration of 22.7 mg/g of fat, much higher than values for cows fed conserved forages. Also of interest is the study of Jiang et al. (42) who found that, with a constant supply of linoleic acid, CLA content could be influenced by the ratio of forage to concentrate. These studies suggest that, given an adequate dietary intake of linoleic acid, dietary constituents that provide ruminal substrates for the optimal growth of bacteria producing linoleic acid isomerase will maximize CLA output. Nevertheless, Kelly et al. (43) reported substantial individual variation (9.9 to 51.7 mg CLA/g of fat) in cows at the same stage of lactation that consumed the same diet and were subjected to the same management regimen. This result suggests additional factors such as individual genetic regulation of rumen microflora may operate (51).

Modifying CLA Concentrations in Human Tissue

Studies have been reviewed (58, 60, 61) showing that human fatty tissue, bile, duodenal juice, blood serum, and breast milk contain CLA and that feeding subjects foods rich in CLA, such as dairy products and ruminant meat, increases their blood CLA concentrations. Parodi (58) proposed that dietary *trans*-11-$C_{18:1}$, the predominant trans monounsaturated fatty acid in milk fat and ruminant tissue fat (56) could be converted to CLA in humans. This hypothesis was based on the observations of Mahfouz et al. (47) and Pollard et al. (67), who demonstrated that Δ9 desaturases from rat liver microsomes can introduce a double bond at the Δ9 position of *trans*-11-$C_{18:1}$ to produce *cis*-9, *trans*-11-$C_{18:2}$ (CLA). Recently, Salminen et al. (73) showed that feeding subjects a diet enriched with trans fatty acids from hydrogenated vegetable oil increased blood CLA levels. An explanation for this CLA increase, no doubt, relates to the presence of *trans*-11-$C_{18:1}$ in the mixture of trans fatty acids. The proportion of dietary *trans*-11-$C_{18:1}$ converted to CLA is not known at this time. Contrary to the above observation, unhydrogenated vegetable oil (safflower) consumption did not increase human blood CLA concentrations (27).

Fogerty et al. (23) noted that breast milk from women of the Hare Krishna religious sect contained twice as much CLA as milk from conventional Australian mothers (11.2 mg/g vs. 5.8 mg/g). This difference was attributed to the large amount of butter and ghee consumed by the Hare Krishna women. Park et al. (55) recently confirmed that CLA concentrations in human milk could be enhanced by increasing the CLA content of the maternal diet. Optimization of CLA concentrations in maternal placental blood and breast milk could protect female neonates from subsequent breast cancer development. This result should be examined with an animal model because Hilakivi-Clarke and colleagues report that diets high in linoleic acid, fed during pregnancy only, increased the risk of developing carcinogen-induced mammary tumors in the mothers (29) and in their female offspring (28).

SPHINGOMYELIN

Sphingomyelin is a phospholipid preferentially located in the outer leaflet of the plasma membrane of most mammalian cells. In bovine milk, phospholipids account for 0.2 to 1.0 g/100 g of total lipids, where they are associated with the milk fat globule membrane. Sphingomyelin represents about one-third of total milk phospholipids (59).

In addition to its structural function in membranes, it is now recognized that sphingomyelin, through its biologically active metabolites, ceramide and sphingosine, plays an important role in transmembrane signal transduction and cell regulation (3, 48). A sphingomyelin pathway of signal transduction has been identified. Extracellular agonists, such as certain cytokines, hormones, and growth factors, stimulate their cell surface receptors to activate sphingomyelinases that cleave sphingomyelin to generate cellular ceramide and phosphocholine. Cera-

mide, in turn, acts as a second messenger for the action of the extracellular agonists, transmitting the signal through multiple downstream targets such as various protein kinases and phosphatases. These, in turn, regulate the function of a number of transcription factors that control the expression of a range of genes responsible for inhibition of cell growth, cell cycle arrest, differentiation, and apoptosis (3, 49). Other agonists, such as platelet-derived growth factor, can trigger further hydrolysis of ceramide to sphingosine. Sphingosine also acts as a second messenger taking part in signaling cascades that modulate cell growth (49). The immune system also depends on ceramide-mediated signaling pathways for the activation and amplification of antigen-specific T- and B-cell clones to combat tumors (3). Because ceramide and sphingosine participate in major antiproliferative pathways of cell regulation that suppress oncogenesis, they have been termed tumor suppressor lipids (59).

Animal Dietary Studies

Dietary triglycerides and glycerophospholipids are rapidly hydrolyzed by pancreatic lipases in the proximal small intestine. On the other hand, because of an inadequate supply of sphingomyelinase in the proximal small intestine, sphingomyelin is digested slowly and incompletely throughout the entire small intestine and colon. Ceramide and sphingosine cleavage products are absorbed by intestinal cells, where they may be utilized locally or resynthesized into sphingolipids for export to the circulation (53, 75). A sphingomyelin-rich sphingolipid diet increased serum phospholipid sphingomyelin levels in rats (35). For these reasons, dietary sphingomyelin and other sphingolipids may be beneficial to the intestine and perhaps other tissues receiving circulating sphingomyelin. Dillehay et al. (13) fed mice milk-derived sphingomyelin after tumor initiation with 1, 2-dimethylhydrazine. Mice fed as little as 0.025 g of sphingomyelin/100 g of diet had a 57% reduction in colon tumor incidence. Mice with supplemented diets also had fewer ACF than did those receiving the control diet. In a larger study with this murine model, Schmelz et al. (76) confirmed that dietary sphingomyelin suppressed colonic ACF formation, but they found sphingomyelin did not reduce tumor incidence or numbers. However, supplementation produced more adenomas rather than the more advanced malignant adenocarcinomas. Confirmation that sphingomyelin, and not a contaminant, in the milk-derived preparation was responsible for the anticarcinogenic action was obtained when a synthetically prepared sphingomyelin suppressed ACF formation (74).

BUTYRIC ACID

Despite its simple chemical nature, butyric acid is a potent antineoplastic agent. It inhibits cell growth and induces differentiation in a wide spectrum of cancer cell lines including those of the breast and colon, where butyric acid can induce apoptosis and may prevent metastases to the liver (48, 59, 90). Butyric acid can also produce maturational effects in noncancerous cells (52). Another important property is the down-regulation of estrogen receptors in breast cancer cells (66). The mode of action for the anticancer properties of butyrate is not completely understood. At the molecular level, it promotes histone acetylation that may benefit DNA repair, suppresses the expression of various proto-oncogenes, and stimulates expression of tumor suppressor genes (59, 60).

Interest in butyric acid as a cancer-preventing agent stems from its role in the colon, where it is produced during bacterial fermentation of dietary fiber and starch. The largely unsuccessful therapeutic use of butyric acid for patients with leukemia is attributed to its short plasma half-life. Because of its therapeutic potential and noncytotoxic nature, butyrate analogues or prodrugs are being developed to extend plasma half-life. One such prodrug is the triacylglycerol tributyrin (52, 59, 60).

Bovine milk fat contains from 7.5 to 13.0 mol/100 mol of butyric acid, which means that about one-third of all milk fat triacylglycerols contains 1 mol of butyric acid. On ingestion, lipase-induced hydrolysis commences in the stomach and is completed on reaching the proximal small intestine. Butyric acid is absorbed by enterocytes and passes to the portal circulation (59, 60). Although the plasma concentration of butyric acid from this source may not be high, it is important to appreciate that physiological effects may be enhanced several-fold due to synergy with further anticarcinogenic components of milk fat and other dietary items. Thus, Tanaka et al. (85) found 1, 25-dihydroxyvitamin D_3 enhanced butyrate-induced differentiation in a human colon cancer cell line. Chen and Breitman (7) reported that retinoic acid, at physiological concentrations, reduced by about one-tenth the concentration of butyrate required to induce differentiation in a human myeloid leukemia cell line. Velazquez et al. (90) noted that a 3-hydroxy-3-methylglutaryl coenzyme A (**HMG CoA**) reductase inhibitor had a synergistic antiproliferative effect in a murine colon cancer cell line when combined with butyrate. Further, Perrin et al. (64) succeeded in

reversing a late stage of carcinogenesis (carcinomatosis) in a rat model of colon cancer using a combination of butyrate and interleukin-2 when neither of these substances alone proved effective. Colon cancer cells are poorly immunogenic; however, the complete regression of tumor masses was attributed, at least in part, to a butyrate-induced increase in immunogenicity of the cancer cells.

The only apparent animal study to examine the effect of dietary butyrate on carcinogenesis, at a site other than the colon, was reported by Yanagi et al. (94). They found that the addition of 6% sodium butyrate to a basal diet containing 20% fat supplied by a margarine made from safflower oil significantly reduced the incidence of DMBA-induced rat mammary carcinomas and adenocarcinomas.

ETHER LIPIDS

Ether lipids are characterized by an ether bond in position *sn*-1 of the glycerol backbone of triacylglycerols and phospholipids. Studies with neoplastic cells of diverse origin show that ether lipids have potent antineoplastic activity at very low concentrations. This phenomenon is a result of their antiproliferative activity and their abilities to induce differentiation and apoptosis, prevent invasion and metastasis, and modulate immune response. The therapeutic potential of ether lipids and derivatives is being determined currently in a number of clinical trials (14, 68).

Milk fat contains small quantities of ether lipids. Studies in rodents and humans showed the 1-*0*-alkyl *sn*-1 glycerols liberated from dietary ether lipids are readily absorbed and transported, without cleavage of the ether bond, to the liver and other organs, where they are used to synthesize membrane phospholipids (59, 60). The use of dietary ether lipids in cancer prevention has not been reported.

VITAMIN D

Milk, as secreted, is not a rich source of vitamin D; thus in North America and Europe, milk is fortified with this vitamin. Vitamin D is sequentially hydroxylated at position 25 in the liver and at the 1-α position in the kidney to form the biologically active hormone 1α-, 25-dihydroxyvitamin D_3. The physiological effects of vitamin D are mediated by vitamin D receptors, which are present in a number of organs and have also been detected in a variety of human cancer cells, including prostate, colon, and breast. In these cancer cells, 1, 25-dihydroxyvitamin D_3 induces biological responses, such as the suppression of growth, induction of apoptosis, and induction of terminal differentiation (22, 82).

The small number of reported animal studies indicate vitamin D and its metabolites may suppress chemically induced skin, colon, and mammary tumors (17, 65). An association between decreased intake of vitamin D and increased risk of prostate and colon cancer is suggested from limited epidemiological evidence (65, 82). In the case of colon cancer, vitamin D may ameliorate the action of calcium.

ANTICARCINOGENS FROM FEED

The dairy cow has the ability to act as an efficient biological extractor and converter of pharmacological compounds from pasture and other feed stuffs ordinarily not suitable for human consumption and to transfer them to milk for human consumption. The best known example is the intake of β-carotene from pasture. During absorption and transport, a portion of β-carotene is converted into vitamin A in the intestine and liver, and both are subsequently transferred to milk (59).

Vitamin A and β-carotene are probably the most widely investigated natural anticarcinogens, which has resulted in numerous reviews (46, 65, 88, 89); their anticarcinogenic action will not be discussed in detail in this review. In brief, epidemiological studies, both prospective and retrospective, consistently find that people eating more fruits and vegetables rich in β-carotene or having higher blood concentrations of β-carotene had a lower risk of developing several types of cancer, especially lung cancer (65, 88, 89). There is, likewise, strong epidemiological evidence for an inverse association between vitamin A (retinol) intake and cancer, particularly skin, urinary, and aerodigestive tract cancer (46, 65). However, it is uncertain whether the epidemiological associations for β-carotene are due to its in vivo conversion to retinol or whether the effect of both is but a surrogate marker for other dietary anticarcinogenic components.

Vitamin A deficiency in animals predisposes to premalignant changes and enhances the development of chemically-induced cancers, including aerodigestive tract and lung, skin, mammary gland, and urinary cancers; however, the results are not always consistent (46, 65). Vitamin A inhibits carcinogenesis in individuals with premalignant lesions and a high risk to develop cancer of the aerodigestive tract (46). Vitamin A and its derivatives are being used in trials for cancer prevention at a number of sites including skin, cervix, breast, and bladder.

In animals, β-carotene suppresses chemically-induced tumors at some sites. The effect is probably

enhanced when combined with other micronutrients, such as vitamins E and C, glutathione, and selenium (88). Although metabolism of β-carotene in animals is notably different from humans, intervention studies with humans suggest β-carotene may protect against cancer of the aerodigestive tract and colon and probably is more effective during the early stages of carcinogenesis (88, 89).

β-Carotene influences carcinogenesis through a number of mechanisms associated with its in vivo conversion to vitamin A. Mediated by nuclear retinoic acid receptors, vitamin A regulates the expression of genes that regulate cell growth and differentiation. Vitamin A also exhibits antioxidant properties and can increase both humoral and cell mediated immune responses. An independent property of β-carotene is its ability to increase the communication of signals between cells. At the extra-hepatic cellular level, β-carotene may convert to vitamin A, which could be important because serum levels of vitamin A are under tight homeostatic control and do not increase with increased intake (89).

Recently, the value of β-carotene in cancer prevention has been questioned. Two large intervention studies found that supplementation of high quantities of β-carotene increased lung cancer risk in high-risk populations of smokers and asbestos-exposed workers. This phenomenon was especially true when vitamin A was also included in the supplement. On the other hand, another major study found β-carotene supplementation did not harm healthy individuals over a long-term period (6). In addition, a recent review of epidemiological studies of the association between vitamin supplementation and cancer risk did not detect an adverse effect for β-carotene in apparently healthy individuals (63).

Cottonseed meal is often used as a protein supplement for dairy cows. The meal contains the polyphenolic pigment gossypol. Gossypol exhibits antineoplastic and antiproliferative action on a variety of human epithelial cancer cell lines (33). Hu et al. (33) demonstrated that milk from cows fed gossypol inhibited the growth of two human breast cancers and an esophageal cancer cell line in rats.

The pasture species alfalfa (lucerne) contains the isoprenoid β-ionone, an end ring analog of β-carotene, which is transferred to milk (96). β-Ionone is a potent suppressor of hepatic HMG CoA reductase activity. This enzyme catalyzes the conversion of HMG CoA to mevalonate, which is required for DNA synthesis and cell proliferation (20). Hypocholesterolemic drugs (statins) that inhibit HMG-CoA reductase activity suppressed the growth of chemically induced and transplanted tumors in animal models (20). β-Ionone inhibited the growth of murine melanoma, rat mammary cancer, and two human breast cancer cell lines (20). Yu et al. (97) reported that dietary β-ionone significantly suppressed DMBA-induced rat mammary tumor incidence and multiplicity while also increasing latency.

It is interesting to note that El-Sohemy and colleagues reported dietary cholesterol significantly inhibited MNU-induced rat mammary tumor development (18) and azoxymethane-induced ACF in the colon of mice (19). In both studies, dietary cholesterol elevated low density lipoprotein (**LDL**) cholesterol levels. The authors believe that LDL-cholesterol entering cells via the LDL-receptor reduces the level of HMG CoA reductase, thus inhibiting endogenous cholesterol biosynthesis in preneoplastic and tumor cells, which inhibits their proliferation.

CONCLUSIONS

This review demonstrates that milk fat contains a number of components with the ability to inhibit carcinogenesis, in vivo with animal models and in vitro for a variety of human cancer cell lines. The author has previously reviewed evidence that shows butter and milk fat are less carcinogenic in animal models than in linoleic acid-rich vegetable oil and margarine (59, 60). For humans, it is difficult to infer a role for milk fat in cancer prevention from epidemiological studies. Milk fat is not consumed as a single entity but is usually accompanied in dairy products by protein and calcium that also have anticarcinogenic properties (62). Further, dairy products form only part of the total diet, which may contain other components that help prevent cancer or components that help cause cancer. Nevertheless, the anticarcinogenic properties of milk fat should be evaluated against the role of total dietary fat in the etiology of cancer. Ecological studies have identified associations between national rates for the major cancers of the breast, colon, and prostate with dietary fat intake (30). However, a metaanalysis of case-control studies (5) and prospective studies (34) found little evidence of a positive association between total dietary fat intake and the risk of breast cancer. Likewise, when the consumption of red meat (24) and total energy intake (83) were taken into account, there was no association between total dietary fat and colon cancer. Indeed, there was even a weak inverse relationship between dairy fat intake and colon cancer (24). Epidemiological studies assessing the risk for breast and colon cancer are now focusing on the type of dietary fat and also on specific fatty acids, because

the various acids have different physiological functions (81, 83).

Nutrient-nutrient interaction is a poorly understood aspect of nutrition science. This review notes, however, that the antiproliferative action of butyric acid is enhanced by synergy with retinoic acid or vitamin D. Future research should investigate the conversion of the major milk fat trans monounsaturated fatty acid, *trans*-11-octadecenoic acid to CLA (58, 73) and the interaction of CLA with other anticarcinogens. The interactions between milk fat anticarcinogens and anticarcinogens from other dietary sources should also be examined. This information should enable an evaluation of the level of the various anticarcinogens required for adequate cancer protection.

It should be possible to increase health-promoting components in milk by modifying the cow's diet. For components already present, such as CLA, studies are progressing satisfactorily. In addition, novel anticarcinogenic components could be introduced to pasture and supplements (for subsequent transfer to milk) by techniques including genetic engineering. This is the challenge for animal science.

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