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Anti-cancer functional milk

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Abstract

Conjugated linoleic acids (CLA) are unique polyunsaturated fatty acids. These naturally occurring compounds have proven to have anti-carcinogenic effects. Both in vivo and in vitro experiments have proven their potential to stop cancer. In this review, we present the numerous effects of CLA isomers on cancer development, such as anti-tumor efficiency, anti-mutagenic activity, and antioxidant activity. Even though most of the in vivo and in vitro studies in this review show that CLA is good at stopping tumor cells from growing and dying, more experiments are needed to find out if it really is a good anti-cancer drug.

Keywords: CLA; dairy cow; milk fat; mechanism

1- Introduction

Fatty acids are critical components of the human body, having metabolic, structural, and maintenance activities. There is significant research interest in fatty acids and their potential health benefits. [1]. Fatty acids are categorized into two main classes, saturated and unsaturated, based on whether they contain double bonds. Saturated fatty acids exclusively consist of single bonds, while unsaturated fatty acids consist of double or triple bonds. Polyunsaturated fatty acids (PUFA) are unsaturated fatty acids that have two or more double bonds. PUFA, or polyunsaturated fatty acids, can be categorized based on their chemical composition. The first category is fatty acids with double bonds, separated by a methylene group (-CH2-), and found in the cis configuration. These fatty acids that exist naturally are referred to as essential fatty acids. Non-conjugated polyunsaturated fatty

acids (PUFA) that are commonly found include linoleic acid, or ω−6 (9 cis, 12 cis-C18:2), which can be found in nuts, seeds, and vegetable oils, and α-linolenic acid, or ω−3 (9 cis, 12 cis, 15 cis-C18:3), which can be found in seeds, plant oils, fish, and shellfish. Double bonds combine to form the second type of PUFA. It includes various positional isomers of fatty acids (9 cis, 11 trans-C18:2, and 10 trans, 12 cis-C18:2), known as conjugated fatty acids (CFA). Conjugated linoleic acids (CLA) are a CFA group containing 18 carbons and 2 conjugated bonds. Conjugated linolenic acids (CLNA) are a subgroup of CLA that have 18 carbons and 3 double bonds. Conjugated eicosapentaenoic acids (CEPA) are another subgroup of CLA that have 20 carbons and 5 double bonds. A well-studied example of conjugated fatty acids is CLA. CLA are naturally occurring isomers of fatty acids present in ruminant animal dietary items [2]. There are 28 known CLA isomers identified with varied locations (ranging from Δ7, Δ9 to Δ12, Δ14) of cis or trans geometry. The main biological isomers of conjugated linoleic acid (CLA) in dairy products are cis-9 and trans-11 (c9 and t11). Together, they make up more than 80% of all CLA isomers. Another isomer, cis-12, trans-10 (t10, c12), is present in certain ruminant fats [3]. The natural derivative of CLA consists of a combination of two isomers: c9, t11, and t10, c12. Bacterial enzymes help change linoleic acid into these isomers by adding hydrogen to it and/or changing the shape of cis-unsaturated fatty acids [4].

The human body primarily obtains conjugated linoleic acid (CLA), a necessary but very small constituent of lipids, from dairy products derived from ruminant animals [5]. The physiological characteristics of CLA have garnered significant interest in recent decades due to their well-documented advantages for health and biological roles. Several studies on animals and cell lines have demonstrated the significant health benefits of CLA, including its ability to reduce the risk of cancer, diabetes, obesity, and atherosclerosis [6]. Booth first identified CLA, or conjugated linoleic acid, in butter fat in 1935. However, one of the earliest recognized health benefits of CLA was its anti-carcinogenic activities, which Pariza et al. discovered in the late 1970s [5]. Fried ground beef provided CLA, an anti-carcinogen [8]. Subsequent research, both in vivo and in vitro, has shown that CLA can effectively promote the inhibition of tumor cell proliferation [7]. Currently, the precise processes by which CLA counteracts cancer are not well understood. Another critical inquiry pertains to the dosage and specific isomer required to achieve the intended beneficial effects. Studies have shown that a blend of CLA can act as an anti-cancer drug by controlling tumor growth through various metabolic pathways. It can also modify lipid peroxidation, cell proliferation, and apoptosis [9]. Several investigations have demonstrated the efficacy of the individual isomers c9 and t11-CLA at optimal dosages, but these studies have not found t10 and c12-CLA to be useful [7]. In contrast to the early research, there have been a few reports of CLA that have not demonstrated any inhibitory effects on cancer and have even encouraged tumor development [10].

2- What is the conjugated linoleic acid?

CLA is a collective term for a group of positional (c8, c10; c9, c11; c10, c12, and c11, c13) and geometric (cis, cis, cis, trans; trans, cis; and trans, trans) isomers of octadecadienoic acid (linoleic acid) with a conjugated double bond system. CLA shares the

same chain length as linoleic acid, but unlike linoleic acid, CLA exhibits conjugated double bonds instead of methylene-separated ones. One single carbon bond separates conjugated bonds instead of two or more bonds. The bonds in CLA can be in either a trans or cis configuration. Researchers first identified it as an anticarcinogenic principal in grilled ground beef [11]. Subsequently, researchers discovered it in numerous other food sources, particularly dairy products [11]. Commercially available CLA sources typically identify up to 18 CLA isomers, with two main isomers (cis-9, trans-11, and trans-10, cis-12) accounting for about 80% of the total CLA. Pariza et al. [12] reported that emerging evidence indicates that the cis-9, trans-11, trans-10, and cis-12 CLA isomers produce different effects. Given the structural differences between these isomers, it is most unlikely that a single biochemical mechanism explains these effects (Pariza et al., 2001).

3- CLA content in milk from different animal sources

The management system has a significant impact on the total CLA content in milk, and milk from the semi-intensive system has the highest levels. The higher concentration of LA in the pasture, known as the primary precursor of CLA, may explain these results. Animal nutrition is the primary factor influencing milk's CLA content. Furthermore, factors such as lactation period, seasonal variation, geographical area, and mastitis presence can all impact the CLA content. According to health claims, grass-fed beef has a 62% lower fat content than grain-fed beef, a 65% lower level of saturated fat, and higher levels of omega-3 fatty acids and CLA. Depending on the cows' diet, CLA concentration in milk typically ranges between 2 and 37 mg/g of fat, with the average CLA content being 4.3 mg/g of fatty acids in cow's milk. Researchers determined the total average CLA content of milk samples to be 1.020%, 0.965%, and 0.961%, respectively. The amount of CLA in food can vary greatly [13].

The larger size of buffalo fat globules, 5 vs. 3.5 µm, is related to the higher amount of fat in buffalo milk: 73.4 ± 9.9 vs. 41.3 ± 3.7 g/kg for cow milk. Buffalo milk contains a significantly higher amount of CLA compared to cow milk. Researchers found that organic buffalo milk has significantly higher CLA levels than regular milk, with 7.3 and 5.5 mg/g fat, respectively. Because and goat milk typically contain more CLA than cow milk due to the semi-extensive nature of the systems used to raise small ruminants. Compared to cow milk fat, goat milk fat contains a greater 56.3% CLA. Goat and ewe milk contain approximately 3.25%–4.2% and 7.1% fat, respectively. According to research, sheep's milk fat contains as much as 2.2% more CLA than goat's milk. Given the same dietary regimen, sheep milk typically contains more CLA than goat milk due to variations in their mammary adipocytes' mRNA. The CLA sheep milk fat content was 2.4% in May, fell to 1.3% in August, and went up to 2.6% in September. The seasonal variations in the pasture are directly responsible for the content of CLA and indirectly responsible for the proportion of CLA in the milk fat. There is not enough information on the CLA concentration in camel milk fat. According to Chamekh et al. [13], camel milk is a rich source of CLA, and the second and third parties, camel milk and colostrum, had the highest concentrations of CLA. Additionally, Abdelsalam, Ali, and Al-Sobayil [14] reported that the stage of lactation had a significant influence on the CLA content of camel milk. However, it's crucial to

remember that other factors, like the camels' diet and the milk's production season, can influence the exact CLA content of camel milk.
Note that these values vary depending on the animal's diet, breed, and milk processing methods. Additionally, the specific CLA isomers present in each type of milk may differ, which can affect their potential health benefits.

4- **Conjugated linoleic acid and antioxidative activity**

Redox homeostasis refers to the equilibrium between oxidants and antioxidants, which is responsible for maintaining the appropriate cellular response to both internal and external stimuli. Nevertheless, an in equilibrium between reactive oxygen species (ROS) and antioxidants triggers the onset of oxidative stress, leading to cellular demise and the emergence of numerous ailments.

In 1990, Ha et al. identified the antioxidant properties of CLA based on in vitro experimental findings. They studied CLA and found that it is a very strong antioxidant, stronger than α-tocopherol and almost as strong as butylated hydroxytoluene (BHT). Pariza and Ha [16] suggested that the antioxidant properties of CLA may contribute to its ability to prevent cancer. Furthermore, Ha et al. [16] and Ip et al. [15] documented that CLA exhibited potent antioxidative effects, suggesting a potential mechanism for its anticarcinogenic capabilities.

However, other investigators have shown that CLA functions more as a pro-oxidant. Van den Berg et al. [17] showed that CLA does not act as an efficient radical scavenger in any way comparable to vitamin E or BHT. Dietary CLA reduces arachidonic acid, linoleic acid, and oleic acid content in fat and shifts the overall fatty acid composition to a more saturated side. Therefore, meats from animals fed CLA may be less susceptible to lipid oxidation, color changes, and volatile production than those from a control diet. Yang et al. [19] used a pure CLA source and found that the entire CLA oxidized rapidly, degrading more than 80% within 110 h at 500 °C in air. The most unstable were four cis-CLA isomers, followed by four cis-trans-CLA isomers. In contrast, four trans-CLA isomers were relatively stable under the same experimental conditions [18]. Flintoff-Dye and Omaye [19] suggested that CLA isomers did not act as efficient antioxidants in vitro.

They discovered that CLA acted as a pro-oxidant and antioxidant, but then reverted to a pro-oxidant as enrichment levels increased. Tsuzuki et al. [20] suggested that alpha-eleostearic acid, a conjugated linolenic acid, has a stronger antitumor effect than CLA, both in vitro and in vivo. They found that the oxidation rate of alpha-eleostearic acid was faster than that of unconjugated fatty acids and CLA. But after alpha-eleostearic acid oxidation, there was less lipid hydroperoxide and thiobarbituric acid reactive substance than CLA.

Ali, Kadir, Ahmad, Yaakub, Zakaria, and Abdullah [21] showed that both isomers of CLA can act as antioxidants. Their ability to scavenge free radicals may help explain their wide range of biological functions. It is also said that CLA is the most powerful antioxidant in milk fat. This is because it works well with other components of milk, such as α-tocopherol, β-carotene, vitamin A and vitamin D3, phospholipids, short-chain saturated fatty acids, vaccenic acid, coenzyme Q10, and ether lipids. It's important to note that CLA changes the redox status of all tissues in different ways and lowers endoplasmic reticulum stress in the

liver and muscles. It also modulates mechanistic links between the actin cytoskeleton, insulin signaling pathway, glucose transport, and inflammation in adipose tissue. Making GSH without lipoperoxidation, lowering the levels of ROS and thiobarbituric acid reactive substances inside cells, and showing a better antioxidant response in cells against H2O2-induced oxidative damage are all things that the t10,c12-CLA isomer does to improve the redox status of mammary epithelial cells in cattle. Through the Nrf2 signaling pathway, c9,t11-CLA improved milk fat production in bovine mammary epithelial cells. This lowered oxidative stress and autophagy, suggesting a natural way to treat mastitis instead of antibiotics. With the increasing demand for natural antioxidants, CLA can be added to a food system to prevent oxidation.

5- Effects of CLA on cancer

The role of CLA in cancer prevention is well documented. CLA is an efficient inhibitor of all stages of carcinogenesis: initiation, promotion, and metastasis, as well as neovascularization or angiogenesis. Numerous studies, both in vitro and in vivo, have demonstrated the anticancer activity of CLA. Animal tests have shown that a mix of CLA isomers and either c9, t11-CLA or t10, c12-CLA isomer alone, at a concentration range of 0.05 to 1% (w/w), stops chemically-induced tumors in the mammary gland, colon, and forestomach neoplasia, as well as the metastasis of cancer cells that have been introduced. Similarly, studies done in a test tube have shown that both c9,t11-CLA and t10,c12-CLA isomers, as well as a mix of isomers, can stop the growth of a number of different cell types, such as prostate cancer cells, colon cancer cells, and breast cancer cells.

Different models of action suggest that the c9, t11, t10, and c12-CLA isomers inhibit cancer. In particular, c9,t11-CLA seems to mainly impact the metabolism of arachidonic acid, lower the expression of 5-lipoxygenase (LOX) and cyclooxygenase (COX), cause lower levels of prostaglandin E2 (PGE2) and thromboxane B2 (TB2), and stop NF-B from activating. This inhibitory activity was attributed to the decreased catalytic activity of IκB kinase (IKK). c9,t11-CLA significantly increased TNF-induced apoptosis in a mouse prostate tumor model, correlating with a reduction in NF-B transcription, NF-B binding activity, and IB phosphorylation. Researchers also demonstrated the influence of CLA on human breast and prostate cancers, demonstrating a clear pro-apoptotic effect. Studies have also shown that t9, t11-CLA, t10, t12-CLA, and other minor isomers can stop the growth of different types of tumor cells, such as human colon cancer and breast cancer [21].

The t10, c12-CLA isomer seems to work preferentially through modulation of apoptosis and cell cycle control; induction of G1 arrest; activation of caspase-3 and caspase-9; induction of p21, p53, and p27; reduction of cyclin D1 and E; decreased CDK2 activity and hyperphosphorylated retinoblastoma (Rb) protein (required for G1 to S phase transition); reduction of anti-apoptotic bcl-2 expression; cleavage of the pro-apoptotic protein Bid; activation of pro-apoptotic protein Bax; reduction of expression of oncogene ErbB3; decreased secretion of insulin-like growth factor II (IGF-II); and induction of pro-apoptotic and anti-tumorigenic nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1). T10,c12-CLA induced apoptosis in mouse mammary tumor cells and simultaneously reduced cell proliferation rate; in those models, neither LA nor c9,t11-CLA

showed pro-apoptotic activity. Notably, the action of t10,c12-CLA was related to reduced levels of the 5-LOX metabolite, 5-hydroxyeicosatetraenoic acid (5-HETE), and adding 5-HETE back to tumor cells reduced the t10,c12-CLA effect on both apoptosis and cell proliferation, indicating that CLA inhibits arachidonic acid metabolism through 5-LOX [23].

6- Conclusions

Conjugated linoleic acid (CLA) has attracted significant international attention in recent decades due to its ability to offer several health advantages, such as preventing cancer, diabetes, inflammation, obesity, and atherosclerosis. Researchers have extensively verified these effects in both living organisms (in vivo) and laboratory settings (in vitro). However, the impact of CLA on people has been significantly less pronounced compared to animals. The precise dosage of CLA that is useful for both animals and humans remains uncertain, and the exact mechanism by which it works is not entirely understood. In addition, there are currently some unknown negative effects that have emerged due to the insufficient number of human trials and the lack of sufficient scientific data to establish a correlation between these adverse effects and the dosage or duration of CLA administration. Investigating potential medication interactions between CLA and various types of medicinal pharmaceuticals commonly used in clinical practice, along with other natural food supplements or functional foods, is crucial. Also, the different CLA isomers have different physiological properties, resulting in different health benefits and risks. This means that everyone reacts differently to CLA. Therefore, it is advisable to consult a healthcare professional or qualified dietitian who can provide personalized guidance based on your specific requirements and health objectives. Studies have linked CLA to numerous health benefits. However, further research is required to gain a comprehensive understanding of its impact on human health and identify the most effective, safest doses of both isomers and the CLA combination with minimal side effects before recommending its use.

Acknowledgment

If necessary.

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