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In Vivo Antitumor Activity of Hydrophilic Arginine-Conjugated Linoleic Acid Complex

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Abstract Although conjugated linoleic acid (CLA) exerted potent antitumor activities in several animal models, application of CLA as a bioactive ingredient has been limited due to its hydrophobicity. This study was designed to determine the antitumor activity of arginine-CLA complex (Arg-CLA), a hydrophilic form of CLA. Mouse forestomach cancer was induced by gavage with benzo(a)pyrene (B(a)P) for 4-weeks prior to Arg-CLA (0.2 and 0.5%) feeding. Complete necropsies were performed to determine the number, size and locations of all the forestomach tumors at 20 weeks post-B(a)P administration. All mice in the B(a)P group developed tumors, and tumor incidences were decreased by 31% and 44% in 0.2% and 0.5% Arg-CLA-fed groups, respectively, whereas no decrease was observed when Arg or corn oil was given alone. Our results suggest that Arg-CLA suppresses mouse forestomach cancer.

Key words: Antitumor, arginine, conjugated linoleic acid, forestomach cancer

Forestomach cancer remains one of the most common diet-related cancers worldwide, particularly in some Asian countries [5]. Dietary modification has been suggested as a practical strategy for the prevention of a variety of cancers. In particular, low intake of smoked, salted and nitrated foods, and high intake of fruits, vegetables, and some dairy products were suggested as preventive factors [11, 13, 17, 21]. Identification and isolation of active antitumor compounds such as conjugated linoleic acid (CLA) may partly explain the potential role of dairy products in the prevention of cancer [4]. CLA is mainly derived from the enzymatic isomerization of linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid) during biohydrogenation of the ruminal microorganisms [20]. It has been shown to inhibit chemically induced tumors in various tissues including the skin [1], the forestomach [7], the mammary [8–10], the prostate [3], and the colon [15, 19] of experimental animals. The antitumor activity of CLA is of special interest in that CLA shows inhibitory effects during different stages of carcinogenesis at relatively low dietary levels [8]. So far, numerous hypotheses have been proposed and tested for antitumor mechanisms of CLA. Ha *et al.* [7] investigated the ability of CLA to inhibit the growth of benzo(a)pyrene (B(a)P)-induced mouse forestomach neoplasia. They noted that CLA may have protective effects against free radical attack. Moreover, Ip *et al.* [8] suggested that CLA could prevent mammary cancer through the modulation of tissue maturation and differentiation. In addition, the mediation of CLA in the eicosanoid synthesis, in a manner similar to that of ω-3 fatty acids, has also been proposed [16]. Thus, on the basis of these investigations, CLA has been given considerable attention as an antitumor agent.

However, the application of CLA has been limited due to its lipid-soluble nature. Arginine (Arg), a substrate of nitric oxide synthase, is known to prevent cardiovascular disease by regulating vascular function and blood pressure homeostasis [18]. Lass *et al.* [14] showed the protective role of Arg against the oxyradical attack by direct chemical interactions. Based on its health-promoting roles, Arg was chosen as a substrate for hydrophilic CLA complex. This study was designed to determine the anti-tumor properties of Arg-CLA, a hydrophilic CLA salt, against mouse forestomach cancer induced by B(a)P.

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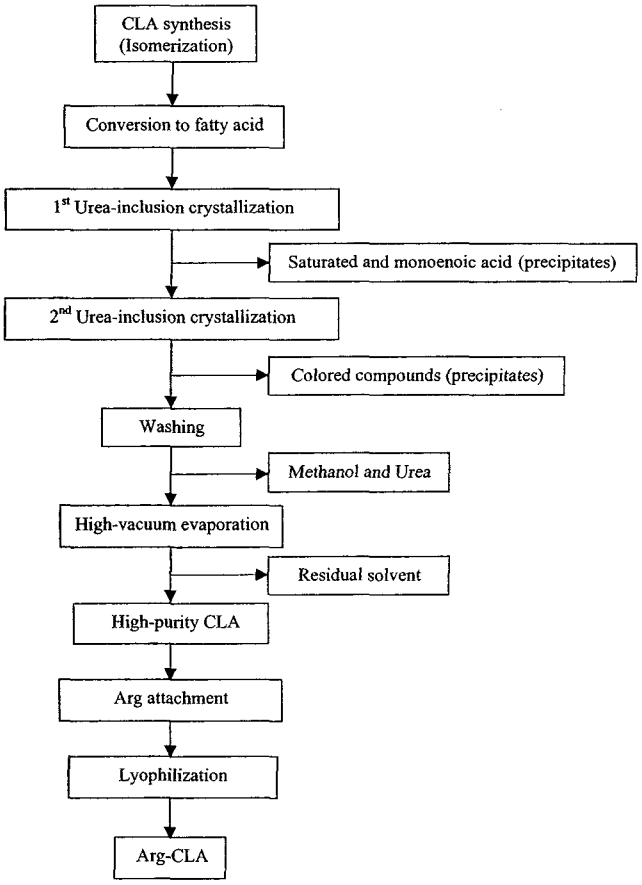

Fig. 1. Preparation of Arg-CLA from safflower oil (12).

Arg-CLA was prepared as described previously [12]. Briefly, 1 kg of Arg was dissolved in 5-l distilled water. The temperature of the reaction mixture was slowly lowered to 0°C, during which the purified CLA dissolved in 95% ethanol was added in small portions while stirring at 500-1,000 rpm until the turbidity of the mixture disappeared. Arg-CLA complex formation was confirmed using infrared spectroscope (FTS 3000 MX, BioRad Co. Hercules, CA, U.S.A.). Overall Arg-CLA preparation procedure is depicted in Fig. 1. Six-week-old female SENCAR mice obtained from Harlan Sprague Dawley (Indianapolis, IN) were housed in plastic cages with a 12 h light/12 h dark cycle at 24°C and 50±10% relative humidity. The ingredients of the experimental diets are shown in Table 1. After one week of feeding, 8 (for control groups) or 16 (for B(a)P treatment groups) mice (20±2 g body weight) were randomized into six groups. The food intake of the mice, which were fed low (0.2%) or high (0.5%) Arg-CLA diets, was adjusted with corn oil to compensate for the difference in dietary energy concentrations. Forestomach cancers were induced by gavage with 1 mg of B(a)P (Fluka Chemical Co., Swiss) in 0.1 ml corn oil twice a week for 4 weeks from the second week. Mice fed with corn oil or Arg without B(a)P treatment served as controls. Body weights and food consumption were recorded weekly, and the experiments were terminated 20 weeks after B(a)P administration. Complete necropsies were performed to determine the number, size, and location of all the forestomach tumors and extragastric lesions. All lesions and representative tissues were fixed in formalin and processed for histopathological examination of the tumors. Differences in tumor incidence were evaluated by Chi-square analysis between dietary groups. Total tumor incidence, body weight, and food intake were compared through the analysis of a variance multiple comparison test.

Table 1. Ingredients of experimental diet.

Ingredients	Amounts (%)
Corn powder	25
Soy cake	25
Wheat bran	10
Wheat powder	10
Egg powder	8
Fish powder	5
Yeast powder	5
Bone powder	1
Mineral mixture	1
Vitamin mixture	0.25
Cod-liver oil	0.2

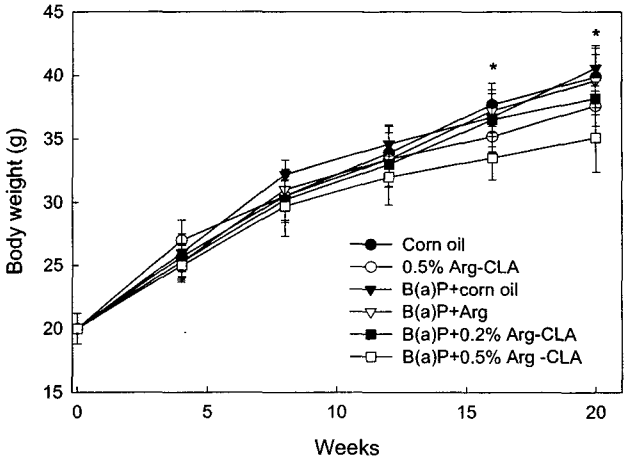

Fig. 2. Body weight of the 6 groups of mice during experimental period.
Mice were weighed every 4 week throughout the experimental period. Each point represents the mean±SE for animals in each group. * represents significant difference between control and 0.5% Arg-CLA-treated group with B(a)P.

The variation in the growth rate of mice fed Arg-CLA was not significant compared with that of the Arg-CLA untreated groups ($P>0.05$). In addition, although the overall food intake of the mice was also not substantially

Table 2. Inhibitory effect of CLA on stomach neoplasia induced by B(a)P.[a]

Groups	No. of animals treated	No. of animals with tumors	Total No. of tumors	Tumor incidence (%)
Corn oil	8	0	0	0
0.5% Arg-CLA	8	0	0	0
B(a)P+corn oil	8	8	17	100†
B(a)P+Arg	16	16	32	100†
B(a)P+0.2% Arg-CLA	16	11	19	69*
B(a)P+0.5% Arg-CLA	16	9	16	56*

[a]Values with different symbols (*, †) in each columns are significantly different ($P<0.05$). All mice groups except corn oil- and Arg-CLA-treated group were treated with B(a)P.

different, the body weights of the 0.5% Arg-CLA-fed mice were lower than those of the control group toward the end of the study period ($P<0.05$, Fig. 2). No toxic effect was observed when Arg-CLA alone was applied throughout the feeding trials. The effects of feeding Arg-CLA with B(a)P treatment are shown in Table 2. When the animals were fed Arg-CLA for 7 weeks after 4 weeks of B(a)P treatment, the tumor incidence was decreased significantly ($P<0.05$). All the mice in the B(a)P groups that were fed corn oil or Arg developed tumors (approximately 2.0 per mouse); however, tumor incidences were 31 and 44% less in the 0.2 and 0.5% Arg-CLA groups, respectively than the control. There was no perceivable difference in total number and size of the tumors among the tumor-bearing mice ($P>0.05$). In addition, no tumor incidence was observed in the mice that were fed corn oil or 0.5% Arg-CLA without B(a)P treatment.

The Arg-CLA feeding in this study was associated with a decrease not only in the number of mice harboring cancer cells but also in the number of tumors in cancerous mice. CLA may have been incorporated into the membrane lipids of neoplastic cells, thereby allowing the cells continuously taken CLA and suppressing cell proliferation. According to Ip *et al.* [9], this continuous intake of CLA was required to achieve the maximal inhibition of tumorigenesis. They noted that 1% CLA in the diet inhibited mammary tumorigenesis induced by methylnitrosourea (MNU; 50 mg/kg body wt) after 8 weeks of feeding. In our study, with the same amount of B(a)P, the incidence of neoplasia was significantly reduced by the uninterrupted feeding of Arg-CLA for 7 weeks. Indeed, significant differences were found between mice fed 0.2% and 0.5% Arg-CLA in both the incidence and the number of tumors. Although the effective level of anti-tumor substances may vary depending on the cell types, dose, and tumor inducers, continuous intake of these compounds, even in small portions, would be required for the effective prevention of chemically-induced cancer.

Interestingly, dietary intake of CLA can moderate the effects of the carcinogenic compounds resulting from pyrolysis such as B(a)P of meat. CLA might provide a natural protective mechanism against undesirable pyrolytic byproducts. Although numerous physiological effects of CLA have been suggested, some concerns have been raised relating to the potential side effects of mega-dose of CLA on insulin resistance and fatty liver [6, 22]. These could be alleviated by Arg-salt formation of CLA, because Arg infusion is known to have a preventive role in insulin resistance by decreasing the total plasma homocysteine concentration [2]. Our results indicate that Arg-CLA, a hydrophilic CLA form, could suppress stomach cancer in vivo. Further study will follow to determine the underlying mechanisms related to the anti-tumor effect of Arg-CLA.

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