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Original Research

Curcumin ameliorates the tumor-enhancing effects of a high-protein diet in an azoxymethane-induced mouse model of colon carcinogenesis

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ABSTRACT

An increasing number of reports suggest that a high-protein diet (HPD) is associated with an increased risk for colorectal cancer (CRC). One of the proposed mechanisms is that an HPD increases the delivery of protein to the colon and generates various toxic metabolites that contribute to colon carcinogenesis. Curcumin was shown to exert significant preventive properties against CRC. We therefore hypothesized that curcumin can reverse the tumor-enhancing effects of an HPD. This study examined the effects of curcumin on the development of azoxymethane (AOM)-induced colorectal tumors in HPD-fed mice. A total of 30 female Balb/c mice were randomly divided into 3 groups: those fed a normal diet (20% casein), those fed an HPD (HPD; 50% casein), and those fed an HPD supplemented with curcumin (HPDC; 0.02% curcumin). The mice were subjected to an AOM–dextran sodium sulfate colon carcinogenesis protocol. Mice in the HPDC group exhibited a significant (40%) reduction in colorectal tumor multiplicity when compared with those in the HPD group. The expression of colonic inflammatory proteins (cyclooxygenase-2 and inducible nitric oxide synthase), the levels of plasma inflammatory markers (nitric oxide and tumor necrosis factor–α), fecal ammonia, short- and branched-chain fatty acid levels, and the rate of colonocyte proliferation were significantly lower in the HPDC than the HPD group. In conclusion, curcumin inhibited the development of colorectal tumors in an AOM-induced mouse model of colon carcinogenesis by attenuating colonic inflammation, proliferation, and toxic metabolite production. Curcumin might be useful in the chemoprevention of CRC in individuals consuming an HPD.

Abbreviations: AOM, azoxymethane; BCFA, branched-chain fatty acid; BrdU, bromodeoxyuridine; COX, cyclooxygenase; CRC, colorectal cancer; DSS, dextran sodium sulfate; HPD, high-protein diet; HPDC, high-protein diet supplemented with curcumin; IL, interleukin; iNOS, inducible nitric oxide synthase; ND, normal diet; NO, nitric oxide; PBS, phosphate-buffered saline; SCFA, short-chain fatty acid; SE, standard errors; TNF-α, tumor necrosis factor–α; TTBS, Tween-20 Tris-buffered saline.

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer death worldwide [1]. There is, however, a wide geographical variation in CRC incidence. Almost 55% of cases occur in more developed regions, with the highest incidence in Australia/New Zealand and the lowest incidence in Western Africa [1]. This difference is thought to be largely attributable to lifestyle and, in particular, to diet. Although it is difficult to isolate and investigate the effects of single dietary components on CRC risk in epidemiological studies because of the complexity of dietary (eg, sugars, alcohols, fruits, and vegetables) and behavioral factors (eg, physical activity, smoking), increasing evidence suggests that the consumption of a Western-style diet with a high fat and/or protein content is correlated with CRC incidence [2,3].

The effect of dietary proteins on CRC risk was shown in animal models of chemical-induced colon carcinogenesis and in in vitro studies. Increased intake of protein in the form of casein, soy, or red meat per se caused DNA damage in colonocytes [4–6], thinning of the colonic mucus layer [4–6], and reduction of brush-border membrane height in rats [7]. Feeding of rats with a high-protein diet (HPD) markedly increased luminal ammonia concentration [7,8], inhibited short-chain fatty acids (SCFAs; acetate, propionate, and butyrate) oxidation [9,10], and stimulated the growth of cancerous cells [11,12]. Moreover, increased bacterial fermentation (putrefaction) of undigested protein delivered to the colon was suggested as being deleterious for the colonic epithelium through increased production of bacterial metabolites derived from amino acids including phenolic and indolic compounds, hydrogen sulfide, and branched-chain fatty acids (BCFAs; isobutyrate, isovalerate, and 2-methylbutyrate) [2,13,14]. In fact, HPD consumption markedly increased protease activities in both the small and large intestines [7] and modified microbiota composition and diversity [15].

Curcumin is the active ingredient of the Indian spice turmeric that is derived from the rhizome of the *Curcuma longa*. Besides its use as a dietary spice, food preservative, and coloring agent, curcumin has also been recognized as a potent herbal medicine against a number of cancers [16–21]. Regarding colon carcinogenesis, increasing evidence has shown its antimutagenic [22], anti-inflammatory [23–25], antiproliferative [26,27], and proapoptotic activities [28,29]. Because orally administered curcumin appears to preferentially distribute to the colorectal mucosa compared with other organs such as the liver [30], studies on the chemopreventive activities of curcumin have naturally focused on colorectal diseases, most notably CRC. In fact, curcumin was found to be one of the most effective chemopreventive agents against CRC [31]. Recent reports on the antitumor effect of curcumin in APC Min mice fed a high-fat diet [32] and in *db/db* obese mice [33] suggest that curcumin might also be effective in the prevention of obesity-associated colon cancer.

No studies have addressed the effects of curcumin on the prevention of colon cancer in animal models fed with an HPD or in humans consuming an HPD. Considering its anti-inflammatory, anticarcinogenic, and probiotic activities [34] in the large bowel, we hypothesized that curcumin might modulate the luminal environment by reducing the production of toxic metabolites and tumor formation caused by feeding mice with an HPD. To test this hypothesis, we first evaluated if dietary curcumin could suppress the tumor-enhancing effects of an HPD in an inflammation-related mouse model of colon carcinogenesis. We then compared gut microbial metabolite production in mice fed with an HPD and those fed with an HPD supplemented with curcumin (HPDC) to evaluate if curcumin changed the luminal environment. Importantly, this is considered a possible mechanism for the chemoprevention property of curcumin.

2. Methods and materials

2.1. Animals and diet

Four-week-old female Balb/c mice were obtained from Koatech Bio Inc (Busan, Korea). All animals were kept in controlled humidity (50% ± 10%), light (12-hour light/dark cycle), and temperature (23°C ± 2°C) conditions and had free access to food and water. The composition of the experimental diet used in this study was based on the AIN-76A formula [35] and is shown in Table 1. The normal diet (ND) contained 20% casein as the protein source, the HPD contained 50% casein, and the HPDC was supplemented with 0.02% curcumin. Food intake was recorded daily. All animal experimentation was approved by the Animal Care and Use Committee at the Catholic University of Daegu (IACUC-2014-042).

2.2. Induction of colon cancer

We used a colitis-related mouse colon carcinogenesis model that was modified from the method of Tanaka et al. [36]. Animals were acclimatized to the animal facility environment for 1 week and then divided into the ND (n = 10), HPD (n = 10), and HPDC (n = 10) groups. To induce colon cancer, the mice were intraperitoneally injected with a single dose of 25 mg/kg

Table 1 – Ingredient composition of the diet fed to mice

Ingredients (g)	ND	HPD	HPDC
Casein	200	500	500
DL-Methionine	3	3	3
Corn starch	150	150	150
Sucrose	500	200	200
Cellulose powder	50	50	50
Corn oil	50	50	50
Mineral Mixture(AIN-76)	35	35	35
Vitamin mix(AIN-76)	10	10	10
Choline bitartrate	2	2	2
tert-Butylhydroquinone	0.01	0.01	0.01
Curcumin	-	-	0.2
Total (g)	1000.01	1000.01	1000.21
% Nutrients of calculated as calories			
Carbohydrate, kcal/kg (%energy)	2600 (67.5)	1400 (36.3)	1400 (36.4)
Protein, kcal/kg (%energy)	800 (20.8)	2000 (51.9)	2000 (51.9)
Fat, kcal/kg (%energy)	450 (11.6)	450 (11.6)	450 (11.6)
Total energy, kcal/kg	3850	3850	3850