Short Communication

Dietary Whey Protein Protects against Azoxymethane-induced Colon Tumors in Male Rats[1]

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Abstract

Epidemiological studies have suggested a relationship between diet and colon cancer incidence. Results from animal studies suggest that whey protein, but not casein protein, may provide protective effects against experimentally induced breast cancer in animals. In the current study, we investigated the effects of casein and whey diets on chemically induced colon cancer in male rats. Pregnant female Sprague Dawley rats (days 3–4 of gestation) were maintained on modified AIN-93G diets formulated with a single protein source of either casein or whey. Life-time exposure to these diets was studied in the F_1 generation (experiment A) or the F_2 generation (experiment B). Male offspring were weaned to the same diets as the dams and were maintained on these diets throughout the study. At age 90 days, all rats received azoxymethane once a week for 2 weeks (s.c., 15 mg/kg). Forty weeks after the last azoxymethane injection, all rats were euthanized, the colon was examined visually for tumors, and each tumor was histologically evaluated. The weights and distribution of all of the tumors were recorded. In experiment A, rats fed the casein diet had a 56% incidence of colon tumors compared with 30% of the rats on whey-based diets ($P < 0.05$). In experiment B, rats fed the casein diet had 50% incidence of colon tumors compared with 29% in the whey group ($P < 0.05$). There were no significant effects of diet on tumor multiplicity or mass. These results suggest that consumption of whey protein-containing diets may reduce the risk of developing colon tumors.

Introduction

Colorectal cancer is the second leading cause of cancer deaths in the United States. The American Cancer Society estimated that during 2000, almost 94,000 people would be diagnosed with colon cancer and that ~48,000 would eventually die of the disease (1). Advances in early detection and surgery have been largely responsible for reducing mortality and morbidity of colon cancer, and our understanding of prevention is increasing.

Epidemiological data suggest that diet is a major factor in the etiology of cancer. Metabolic phenotype, the Western-style diet (low dietary fiber and high levels of fat and red meat), and cooking techniques (*e.g.*, charbroiling or overcooking) are risk factors for developing colon cancer (2, 3). For example, people who consume relatively high levels of well-cooked pan-fried or charred meats and who also have rapid metabolic phenotypes for cytochrome P4501A2 and slow metabolic phenotypes for acetyltransferase may be at increased risk for colon cancer (4). Thus, reduced consumption of charred meats, especially in people who may be genetically predisposed to greater colorectal cancer risk because of their metabolic phenotype, may be important in lowering risk of colon cancer.

Moreover, epidemiological and animal studies suggest that diets low in animal fat and high in fruits, vegetables, grains, and legumes may protect against colon cancer. For example, diets containing soybeans and soybean-based products may reduce the risk of certain types of cancer, including breast, prostate, and colon cancer (5, 6). Data obtained from studies of Japanese subjects point to lower colon cancer incidence in areas with high tofu consumption (7). Furthermore, several animal studies have suggested that diets containing certain vegetables, grains, or specific phytochemicals reduce the risks of experimentally induced colon cancer (8–10).

In addition to the protective effects of certain phytochemicals, bovine milk products may exert inhibitory effects on the growth of several tumor types (11). An antitumor activity of these dairy products has been attributed to a class of proteins that represent 20% of the total milk protein, the whey fraction (12). Recently, Tsuda *et al.* (13) reported that the major whey protein component, bovine lactoferrin, reduced the incidence and multiplicity of colon carcinoma in male rats. GSH[3] concentrations in a number of tissues have been reported to increase in rats fed whey protein, and this is thought to be attributable to relatively high levels of γ-glutamylcysteine groups, which serve as substrate for glutathione synthetase (11). γ-Glutamylcysteine groups are considered extremely rare in edible proteins, with whey protein being one of the few such proteins containing the glutamylcysteine disulfide link (14). Increased tissue concentrations of GSH would be predicted to have a protective effect because elevated antioxidant capacity would favor decreased mutagenicity.

Recently, our laboratory has demonstrated that AIN-93G diets, which are rich in whey protein, reduced the incidence of chemically induced mammary tumors by 38–46% compared with casein in female Sprague Dawley rats (6). The present study was conducted to determine the possible preventive effects of lifetime exposure to whey proteins on AOM-induced colon tumors in male Sprague Dawley rats.

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[3] The abbreviations used are: GSH, glutathione; AOM, azoxymethane.

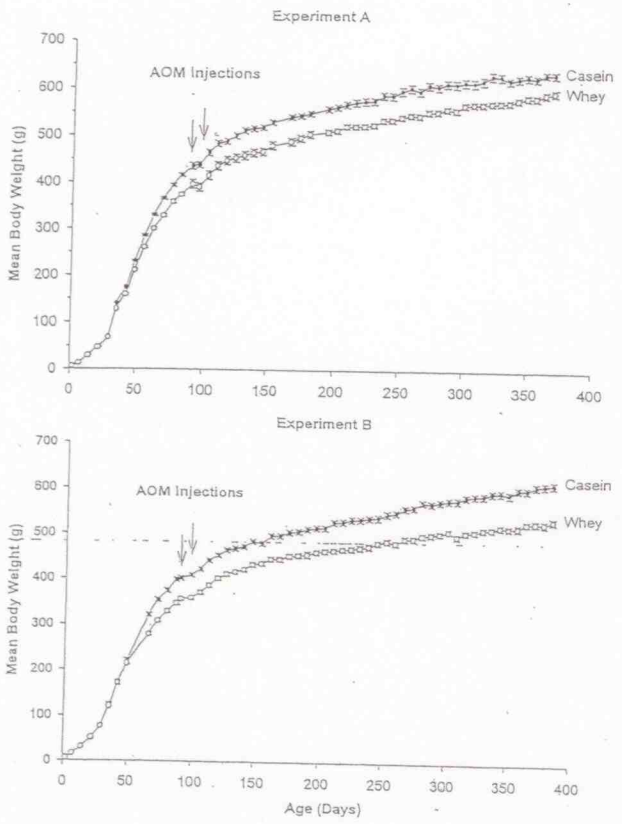

Fig. 1. Average body weights of male rats during the study. At age 90 days, rats received two s.c. injections of AOM (15 mg/kg) as indicated by the *arrows*. In experiment A (*top panel*), 32 rats were fed casein and 42 rats were fed whey diets. In experiment B (*bottom panel*), 42 rats were fed casein and 49 rats were fed whey diets. Data are presented as means; *bars*, ± SE.

Materials and Methods

Adult breeder female and male Sprague Dawley rats, purchased from Harlan Industries (Indianapolis, IN), were housed individually in polycarbonate cages and allowed *ad libitum* access to water and pelleted food. All rats were housed in an American Association for Accreditation of Laboratory Animal Care-approved animal facility.

Two experiments were performed. In experiment A, pregnant female Sprague Dawley rats (gestation day 4) were randomly assigned to one of two groups and fed a modified AIN-93G diet (15) in which corn oil was substituted for soybean oil and the protein source of either casein or whey (New Zealand Milk Products, Santa Rosa, CA). Amino acids were added to both diets to equalize the essential amino acids. Male offspring (F_1) were weaned to the same diets as their dams and were maintained on these diets throughout the study.

In experiment B, female rats were maintained on the diets described above for 4 weeks prior to breeding, and the offspring from these dams were weaned to the same diet as their mothers. Male and female offspring from different parents within a diet group were selected at random and mated to form the F_2 generation. The F_2 generation was studied to simulate people consuming the same basic diet for generations.

At age 90 days, all male offspring from experiments A and B received s.c. injections of 15 mg/kg AOM (Ash Stevens, Detroit, MI) in saline once a week for 2 weeks. All procedures were approved by the Institutional Animal Care and Use Committee at University of Arkansas for Medical Sciences, and AOM handling was in accordance with manufacturing and institutional guidelines. Rats were weighed weekly and observed daily during the first 30 days for signs of toxicity (*i.e.*, fecal blood, altered fur coat appearance, anemia, and body weight gains). Forty weeks after the last AOM injection, all rats were euthanized, and the colon (cecum to anus) was divided into two equal segments (proximal and distal), opened longitudinally, washed free of contents with ice-cold saline, and examined visually for tumors. The locations, weights, and distribution of all tumors were recorded. A representative section of each tumor was fixed in 10% neutral-buffered formalin. Sections (5 μm) of the paraffin-embedded tumors were stained with H&E for histological analysis.

Table I Incidence and histology of AOM-induced colonic tumors in male rats

	Experiment A				Experiment B			
	Casein		Whey		Casein		Whey	
	n[a]	%[b]	n	%	n	%	n	%
Rats per group	32	100	47	100	42	100	49	100
Rats with tumors								
Entire colon	18	56	14[c]	30	21	50	14[c]	29
Proximal	8	25	5	11	13	31	6[c]	12
Distal	12	38	9[d]	21	13	31	8	16
Tumor type								
Invasive adenocarcinoma								
Entire colon	7	22	7	15	13	31	9	18
Proximal	5	16	3	6	8	19	4	8
Distal	3	9	4	9	7	17	5	10
Benign								
Entire colon	11	35	7	15	8	19	5	10
Proximal	3	9	2	4	5	12	2	4
Distal	9	29	5	11	6	14	3	6

[a] Number of rats per group per experiment.
[b] $\frac{\text{Number of rats}}{\text{Number of rats per group}} \times 100$.
[c,d] Compared with casein within the same experiment: [c] $P < 0.05$; [d] $P < 0.01$.

Pathology. All tumors were evaluated in a blinded protocol by an American College of Pathology-certified pathologist (S. K.) and classified.

GSH Concentrations. After the lifetime feeding of casein or whey, male (age, 65 days; $n = 5$) and female (age, 50 days; $n = 5$) rats were euthanized. The livers were removed, and cytosols were prepared by using the method of Chipman and Walker (16). Soluble protein was assessed using the Coomassie Brilliant Blue assay (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions, and cytosolic GSH concentrations were determined using a colorimetric kit (Oxis Int., Inc., Minneapolis, MN) according to the manufacturer's instructions.

Statistical Analysis. Fisher's exact test was used to compare the percentage of rats with tumors in each treatment group. The nonparametric Mann-Whitney U test was used for comparing tumor multiplicity and weight of tumors. GSH levels were compared by t test. Statistical significance was set at $P < 0.05$. The technique described by Fisher (17) was used to combine the probabilities from the two experiments.

Results

Body Weight. Fig. 1 demonstrates that rats fed both diets had excellent body weight gains throughout the study. Consistent