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Inhibitory effect of whole oat on aberrant crypt foci formation and colon tumor growth in ICR and BALB/c mice

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ABSTRACT

Recently, the incidence of colon cancer has been rapidly increasing in previously low-risk countries other than the Western world. Since dietary factors are thought to be key components involved in high risk colon cancer, the current trend for colon cancer prevention is toward dietary intervention. To explore if whole oat functions as a chemoprevention agent, an inflammation-related mouse colon cancer model, initiated with 1, 2-dimethylhydrazine (DMH), followed by dextran sodium sulfate (DSS), was performed to evaluate the preventive effect of whole oat containing diets. The result indicated middle and high dose whole oat diets significantly reduced the number of aberrant crypt foci (ACF) as well as colon tumors. Further, human colon carcinoma cells were subcutaneously inoculated into BALB/cAnNg-Foxn1 nude mice to measure the growth inhibition on whole oat diets. Low, middle and high dose whole oat diets significantly decreased the tumor volumes by 13%, 17% and 43%, respectively, indicating a dose dependent inhibitory effect. Meanwhile, 38% and 54% reductions in tumor weights were observed in middle and high dose whole oat diets. Together, the evidence suggests whole oat helps protect against colon cancer development and could be a good chemoprevention agent taken as a daily supplement.

1. Introduction

It is well-known both genetic and environmental factors are involved in cancer development (Barros and Offenbacher, 2009). For colon cancer, only about 5% has a hereditary component; conversely, the overwhelming majority is sporadic. Sporadic colon cancer results from genetic alternations in colonic mucosa by long term exposure to internal and external carcinogens (Feagins et al., 2009). Epidemiological research indicates the risk of genetic and sporadic colon cancer is associated with dietary habits (Giovannucci et al., 1994; White, 1992; Willett et al., 1990). Thus, cancer prevention has tended to diet-based intervention considering safety and economics. Several epidemiological and animal model studies have shown dietary fiber can reduce cancer risk. However, the association between colon cancer with dietary fiber remains puzzling (Bode and Dong, 2009; Lippman, 2009). The effect of dietary fiber on the reduction of colon cancer has been attributed to increased fecal bulk, dilution of potential carcinogenic compounds and the fermentation by colonic anaerobic bacteria to produce short-chain fatty acids (Bordonaro et al., 2008; Reddy et al., 1989, 2000).

Oats are an excellent resource of dietary fiber, particularly β-glucan that has been linked to prevention of diabetes and cardiovascular disorders (Sadiq Butt et al., 2008; Slavin, 2003). Interestingly, oat fiber fraction has been demonstrated to produce the highest amount of SCFA among dietary fiber fractions from corn, oat and wheat by in vitro fermentation. Further, studies of daily supplementation with β-glucan-enriched oat bran have shown increased SCFA in healthy subjects (Hallert et al., 2003; Nilsson et al., 2008). Actually, whole oat consists of bran, endosperm and germ. All contain high amounts of valuable nutrients. As well as dietary fiber, vitamins, antioxidant, phenolic compounds and minerals, being important nutrient components, have been suggested to possess potent anticancer activities (Bode and Dong,

Abbreviations: ACF, aberrant crypt foci; DMH, 1,2-dimethylhydrazine; DSS, dextran sodium sulfate.

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2009; Sadiq Butt et al., 2008). Incorporating all three parts of the whole oat should improve not only nutrition but also act as a chemoprevention reagent against colon cancer. Therefore, we proposed whole oat, retaining all helpful components, may exert a synergistic effect of abundant protective compounds on colon cancer prevention.

To clarify whether whole oat is effective at protecting against tumor formation, a well-established inflammation-related mouse colon cancer model initiated with 1,2-dimethylhydrazine (DMH) followed by dextran sodium sulfate (DSS) stimulation (Kohno et al., 2005; Onose et al., 2003; Wang et al., 2004) was used to mimic the effect of colon cancer prevention in humans. Besides, a tumor xenograft assay was performed to access the inhibition of colon tumor growth by whole oat. In this study, we provided evidence strongly supporting the claim whole oat significantly inhibits aberrant crypt foci (ACF) formation and colon tumor growth in experimental mice, suggesting whole oat could be a good chemoprevention food taken as a daily supplement.

2. Experimental

2.1. Animals and diets

Four-week-old ICR mice (National laboratory animal center, Taiwan) were housed in cages and maintained in a temperature (22 ± 2 °C) humidity (50 ± 5 °C)-controlled animal facility with a 12 h light–dark cycle and allowed ad libitum access to water. The compositions of the control diet containing low dietary fiber and experimental diets containing whole oat (low, middle and high dose, respectively) are shown in Table 1A and B. The formulations of all experimental diets were based on the AIN-76A diet provided by STANDARD Foods Co., Taiwan. The experiments were carried out in accordance with the Guide for Animal Experimentation in the Laboratory Animal Center, Chung Shan Medical University, Taiwan.

2.2. ACF detection and colon pathology

The inflammation-related mouse colon cancer model was performed as described previously with some modification (Kohno et al., 2005; Onose et al., 2003; Wang et al., 2004). A total of 50 ICR mice were randomly divided into group A–E (10 mice per group). Group B–E received a single subcutaneous injection of DMH (20 mg/kg body weight, Sigma–Aldrich Co., USA), and were then fed with 2% (w/v) of DSS (MP Biomedicals, LLC., French) in drinking water for 1 week. Conversely, Group A received a saline vehicle alone as the normal control. Groups A and B were given the control diet. However, Groups C–E were pre-fed with 8g of daily experimental diets containing low, middle, and high dose whole oat, respectively for 1 week before DMH/DSS treatment. The dietary regimens were continued until the end of the study. The body weights of all mice were recorded every 2 weeks. All groups were sacrificed 25 weeks after injection of DMH. The entire colon was removed, opened along the longitudinal median axis, fixed overnight in a 10% paraformaldehyde and then stained with 0.2% methylene blue. The colon was divided into three segments referred to as the proximal, middle and distal colon. The numbers of ACF were counted and the incidence of ACF was expressed as ACF per mouse. The fixed segments of colon were further embedded in paraffin, and then sectioned and stained with henatoxylin and eosin (HE) for microscopic examination.

Table 1
(A) Ingredient of whole oat (per gram), (B) Composition of experimental diets (g/kg diet).

(A)	
Whole oat	
Calorific capacity (kcal)	3.55
Protein (g)	0.13
Lipids (g)	0.08
Carbonhydrate (g)	0.67
Fiber (g)	0.10
β-glucan (g)	0.04

(B)				
Ingredients	Control	Low dose	Middle dose	High dose
Whole oat	0	75	150	225
Corn starch	672	613	554	494
Casein	200	190	180	171
Soybean oil	50	44	38	32
Cellulose	28	28	28	28
AIN-76 mineral mixture	35	35	35	35
AIN-76 vitamin mixture	10	10	10	10
DL-methionine	3	3	3	3
choline bitartrate	2	2	2	2

Experimental diets were from STANDARD Foods Co, Taiwan.

2.3. Xenograft tumor assay

Five-week-old BALB/cAnNg-Foxn1 nude mice were obtained from the national laboratory animal center, Taiwan. 5×10^6 of Lovo cells, human colon carcinoma cell line, in 400 μl matrigel were implanted into the right flank of the nude mice. After inoculation, the mice were randomly divided into Group A–D (5 mice per group) and fed 5 g of the daily control diet and experimental diets containing low, middle and high dose whole oat, respectively. Tumor volume was measured for 7–63 days. At the end of the observational period, the mice were sacrificed and tumor xenografts were dissected for final volume and wet weight measurement.

2.4. Statistical analysis

Body weight, ACF incidence, tumor volume and tumor weight were determined for all mice fed with the control diet or experimental diets containing different dose whole oat. All data was analyzed by Student's t test. The difference was considered statistically significant at $P < 0.05$ and $P < 0.01$.

3. Results

3.1. Whole oat reduces the number of ACF and colon tumor in ICR mice

The body weights of Groups A–E were comparable throughout the study and showed no significant difference among all groups (Table 2, $P > 0.05$). In saline-treated animals, no ACF was detected in the entire colon (Table 3, Group A). On the contrary, ACF incidence (percent of mice with ACF) was 90% in DMH/DSS-treated mice and the distribution of ACF was mostly located in the middle and distal colon (Table 3, Group B). The total numbers of ACF in mice on the control diet or experimental diets containing low, middle and high dose whole oat were 20 ± 3, 14 ± 4, 14 ± 2 and 12 ± 2, respectively, indicating middle and high dose whole oat diets significantly decreased ACF formation (Table 3, Group D and E, $P < 0.05$ compared with control). Notably, the total numbers of colon tumors in mice on the control diet or experimental diets containing low, middle and high dose whole oat were 10, 2, 0 and 0, respectively. This showed whole oat significantly inhibited colon tumor in a dose dependent manner (Table 4, Group B–E). Together, the data indicates whole oats could inhibit ACF formation and colon tumor development in DMH/DSS- induced ICR mice.