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Anticancer Effects of Isoflavones and Probiotics in Colon Cancer Induced Rats

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INTRODUCTION

Colon cancer is the fourth most common incident cancer following by breast, lung, and prostate and is the second leading cause of cancer deaths in the US. Colon cancer is essentially the only cancer that occurs with approximately equal frequency in men and women (Shike et al. 1990).

Epidemiological studies demonstrate that people consuming large amounts of legumes, fruits, and vegetables have lower incidences of cancer (Boyle et al. 1985). A large number of Asian people who highly consume soy based products in their diets, is known to have lower incidences of breast and colon cancers when compared to the U.S. and some European countries that have high fat and very low soy consumption (Messina 1995). The relatively high amount of soy consumed by Asians could be responsible for the differences in cancer mortality (Adlercreutz et al. 1995). Soy contains a variety of phytochemical compounds that are potential anticarcinogens such as acid phenolics, isoflavones, phytates, phytosterols, protease inhibitors and saponins (Kennedy 1995, Tsukamoto et al. 1995). The presence of these compounds makes soy a prime target for the investigation of chemopreventive action.

Aberrant crypt foci (ACF) are recognized as early preneoplastic lesions. They have consistently been observed in experimentally induced colon carcinogenesis in laboratory animals. Pretlow et al. (1992) have also shown that these lesions are present in the colonic mucosa of patients with colon cancer and have suggested that aberrant crypts are putative precursor lesions from which adenoma and carcinomas develop in the colon. ACF express mutations in the apc gene and ras oncogene that appear to be biomarkers of colon cancer development.

There are at least 400 species and over 10^4 bacteria amounting to several hundred grams of bacterial mass in the human intestinal tract. Among them, anaerobic organisms such as Bacteroides, Bifidobacterium, and Eubacterium are the most predominant in the large intestine and facultative aerobic E. coli and Streptococcus are more prevalent in the small intestine. After the weaning period, Bacteroides outnumber Bifidobacterium and comprise the greatest population throughout our life time.

Genus Bifidobacterium is a nonpathogenic, gram positive and anaerobic bacteria which inhabits intestinal tracts of humans and animals. In brest-fed infants, bifidobacteria comprise more than 90% of the bacterial population, however their numbers gradually decrease over the life time of the host. Bifidobacterium sp. Are used in commercial fermented dairy products and have been suggested to exert health promoting effects on the host by maintaing intestinal microflora balances, improving lactose tolerance, reducing serum cholesterol levels, increasing the synthesis of vitamins, and aiding

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anticarcinogenic activity.

Therefore, this study was designed to investigate the inhibitory activity of *bifidobacterium* and soymilk powder against AOM-induced ACF formation and a modulation NOS activities in the colon of male F344 rats. The goal of this study was to determine if *bifidobacterium* and soymilk powder is conceivably a chemopreventive effect against colon cancer.

MATERIALS AND METHODS

1) Animals

Two hundred 21-day-old male Fisher 344 rats (average body weight=40 g) were purchased from Charles River Breeding Laboratories in Wilmington, MA 01887, U.S.A. Upon receipt, they were housed in wire-topped plastic cages, six animals per cage with laboratory-grade pine shavings as bedding. They were injected with AOM (Azoxymethane, 15 mg AOM/kg body weight) twice in order to initiate colon cancer. First injection was on the next day between 8 : 00 and 10 : 00 am after arrival and second one was on the next week after the first injection. All animals were received a standard diet and tap water ad libitum and were kept on an alternating 12 hour-light- dark cycle, temperature of 20～24°C, and relative humidity of 50～70%. Following a month acclimatization period, the rats were matched for body weights and allocated into 5 groups (n=30 rats/group).

2) Weights

Animals were weighed every week for 8 weeks.

3) Diets

The diet composition was based on the AIN 93 G (Philip et al. 1993). The diet composition was based on AIN-93 G. The all diets have the same proportion of 16.68% fat, 18.9% protein and 58% carbohydrates. All the rats were fed their diets with drinking water for 8 weeks.

4) Dietary soy isoflavone extraction and quantitation

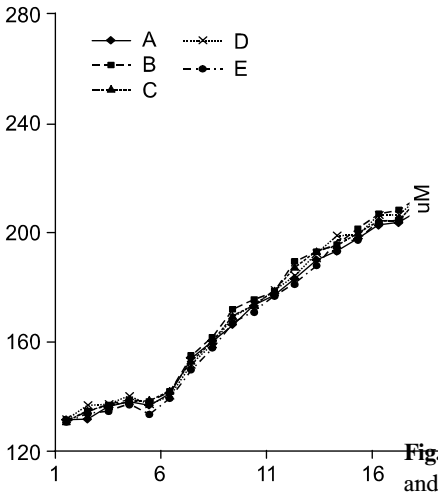
Diet sample (3 g±0.0001) put into a 100 ml beaker. 20 ml of 0.1 N HCL was added and mixed to wet the sample. 30 ml of absolute methanol was added and the sample was sonicated for 20 min. Quantitatively the mixture was transferred to a 100 ml volumetric flask. The beaker was rinsed with methanol and brought to volume with methanol and shaken to mix well. 1 ml or so of the extract was centrifuged at about 10,000-x g for 5 min. The sample was injected into the HPLC with a C18 column (46×25 cm, 5 micron packing) and gradient elution.

5) ACF analysis

After a minimum of 24 hours in buffered formalin, the opened colons were cut into 2 cm segments, starting at the anus, for the next 5～10 min they were placed in a Petri dish containing 0.2% methylene blue in Krebs Ringer solution. They were then placed, mucosal side up, on a microscope slide and observed through a light microscope. ACF were recorded according to standard procedures that are being used routinely in my laboratory. Aberrant crypts were distinguished from the surrounding normal crypts by their increased size, significantly increased distance from lamina to basal surface of cells and the easily discemible pericryptal zone. The parameters assessed were occurrence and multiplicity of aberrant crypts. All colons were scored by one observer without knowing the identity of agents under study, scores were checked at random by a second observer.

6) Assay of NOS and iNOS activity

Conversion of L-arginine to L-citulline was measured by a modification of Ambs et al. (1998).



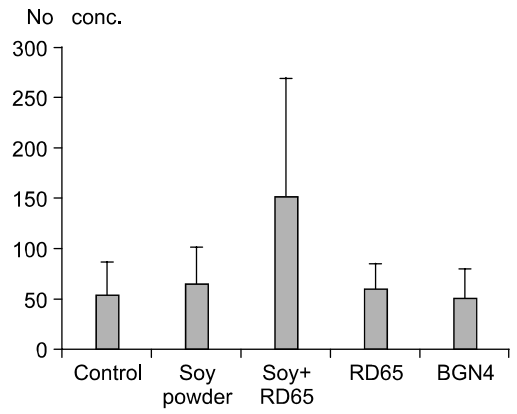


Fig. 2. Effects of probiotic and soy milk on NO (nitrite and nitrate) concentration in plasma of rats.

Fig. 1. Average body weight of male F344 rats treated with AOM. A: control, B: soy powder, C: soy+RD65, D: RD65, E: BGN4.

RESULTS AND DISCUSSION

Animal body weights were shown in Fig. 1. The body weights were increased by duration but body weights in all the groups were slightly higher than that in control group. The content of NOx in plasma did not differ among all the groups (Fig. 2). The picture of colon was shown in Fig. 3 indicating a is a normal colon tissue and b is ACF having three AC per 1 ACF. Fig. 4 showed the average counting number of ACF. All the experimental groups had significantly lower number of ACF compared to control group ($p < 0.05$).

Soy and probiotics are very well known in Asia and western countries as a traditional food, respectively. Also, they have been studied the functions and mechanisms as a functional foods. Recently, Asians change in their eating habits into western style leading to increased morbidity and mortality for colon cancer. So, this study has been attempted to find out the inhibition effect and mechanism of probiotic microflora on colon cancer influence. Soy has widely used as an important source of functional food. Particularly, genestein has an inhibition effect in. Toshihisa et al showed that soymilk that fermented *bifidobacterium* inhibited the breast cancer. Zakia et al observed that fermented milk improved the intestinal microflora condition Soy milk can maintain the activity of the probiotic and improve the health. It can be concluded that probiotic hydrolysis the soymilk then it make easy to digest and absorption. However, these kinds of studies did not perform domestically and it is not clearly identified the colon cancer mechanism of the initial progression and inhibition stage. This study performed to

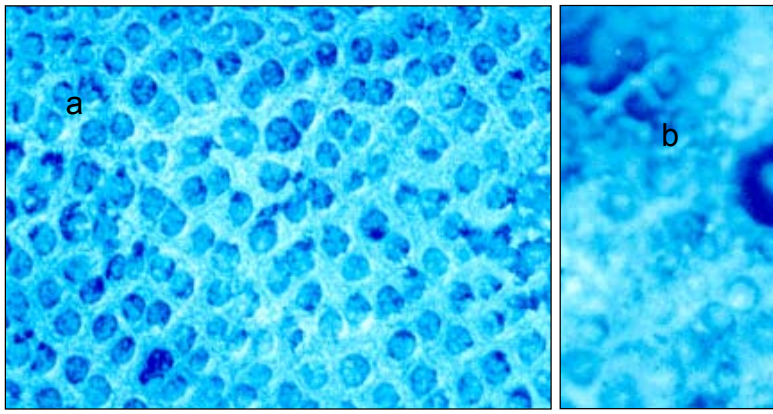


Fig. 3. Topographic view (a: topographic view of colon tissue, b: topographic v
AC).

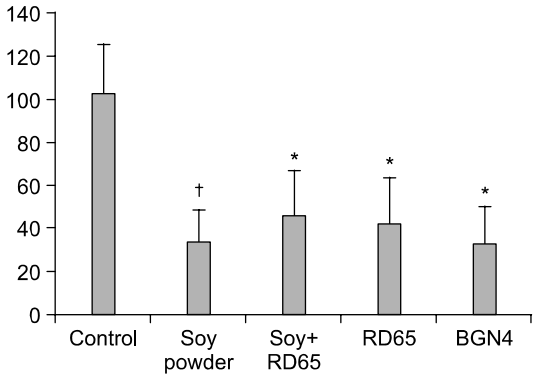


Fig. 4. Effects of probiotic and soy powder on aberrant crypt foci in the colon of rats treated with AOM. *$p < 0.05$ † $p < 0.01$.

find out whether the probiotics and soymilk that fermented by using the probiotic can inhibit an initiation stage of the colon cancer that induced by AOM injection. Linda et al showed the probiotic could significantly inhibit the colon cancer by lowered the cecum pH and ACF in colon cancer induced rats Ae-son Om et al and Reza Hakkak et al studies proved that the soy also could have an inhibitory effect on colon cancer by lowering the ACF in colon cancer induced rats. For instance, the soy powder group has significantly less ACF compare with the control group ($p < 0.01$). Therefore, it showed that colon cancer progression was inhibited by soy administration. Other groups also significantly decreased the ACF compared with the control group ($p < 0.05$).

Lei Lu et al and Edurado J. et al studies reported that the probiotic could improve activity of the intestinal immune function. However this study showed that the activity of the macrophage improved by increasing the level of NO than control as well as expressing the activity of the iNOS. The level of the NO and iNOS expressed high in the probiotic administrated group. It is probably because the probiotic directly involved in intestinal immune function. The results suggest that probiotics and soymilk seem to inhibit the initiation stage of the colon cancer and lowered the level of the intestinal pH. And also it may be improved the immune function by increasing the macrophage activation.

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