

Therapeutic effects of cow and soy milk yogurt consumption on chemically induced colon tumors in rats.

Denise L. Dahle and Peter J. Schaaf

Faculty Sponsors: Margaret A. Maher and S. N. Rajagopal,
Departments of Biology/Microbiology

ABSTRACT

Lactic acid bacteria (lab), used in the production of fermented dairy products, have been reported to have a positive effect on immune system function. In this study cow milk and soy milk yogurt prepared with commercial yogurt starter cultures combining *Lactobacillus delbrueckii* sub sp. *bulgaricus*, *Streptococcus salivarius* sub sp. *thermophilus*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus* were mixed with nutritionally balanced powdered diet and fed to male Sprague-Dawley rats. The rats were injected weekly with 1,2-dimethylhydrazine (DMH), a known colon carcinogen. Both experimental groups contained 7 animals. In addition, there was a biological reference group (n=7) that received no yogurt and no DMH and a control group (n=7) that received DMH, but no yogurt. Food intake and food spillage were recorded daily. Body weight was recorded weekly. Fecal enumeration of lactic acid bacteria was completed at -3, +5 and +11 weeks. Colon tumor number was measured at +11 weeks. The above procedures allowed us to assess if immune protection, after carcinogenic challenge, was provided by LAB supplementation in yogurt made from various milk sources. At the end of the study, aberrant crypts in the distal 10 cm of the colon were 48.58 ± 10.37 for the control group, 41.27 ± 7.13 for the soy group, 40.96 ± 8.73 for the cow group, and 0 for the reference group. There were no significant ($p < 0.05$) differences among DMH-treated groups. However, a trend was observed toward a probiotic effect of LAB in preventing colon carcinogenesis. The dosage of DMH used in this study was on the high end of the literature values and using less DMH may allow for more sensitive detection of LAB effects.

INTRODUCTION

Cancer is the second leading cause of death due to disease in the United States. Colorectal cancers number 152,000 new cases diagnosed and 52,000 deaths each year in the U.S. People over the age of 50 are more susceptible to colorectal cancer and successful treatment is dependent upon early detection of small, localized mucosal tumors (Martini et al., 1997).

The number and quantity of carcinogens (cancer causing agents) in the environment are steadily increasing. Technical advances in polymer chemistry and long-standing industries, such as paper milling and agriculture, have contributed carcinogen-filled effluents that may or may not be cleansed from the drinking water during treatment. Moreover, many potential carcinogens are purposely consumed, such as charbroiled steaks or foods high in preservatives (Keith, 1997).

There are many preventative measures for reducing the risk of developing cancer. One of these prevention methods is to closely supervise diet, excluding foods that are harmful and including foods that may provide protection. Thus, the field of functional foods is growing. The term "functional" has been applied to define the physiological benefits from food beyond the basic nutritional content. For instance, soy protein is being studied for its therapeutic benefits in heart disease and osteoporosis. Moreover, several anticarcinogens have been identified in soybeans, such as protease inhibitors, saponins, and isoflavones. Soy is the only significant dietary source of isoflavones, compounds that may reduce the risk of estrogen-dependent cancers (Hasler, 1998).

Probiotics are defined as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance" (Fuller, 1994). There are suspected probiotic effects of lactic acid bacteria (LAB), found in fermented dairy products such as yogurt, to the gastrointestinal tract. One company is marketing a new product named Culturelle® for its probiotic effects. This product contains *Lactobacillus* in capsules that have been suggested to form a protective barrier in the intestinal tract. These bacteria reportedly can survive the numerous acids in the stomach and adhere to the intestinal lining. This purposeful consumption of LAB for health has been popular in Europe and other parts of the world and is just becoming common in the U.S.

Dietary components have been shown to be major contributors to aberrant crypt growth in gastrointestinal regions. Incidence of colon tumors produced by 1,2-dimethylhydrazine (DMH) was directly proportional to the dietary fat content ranging from 30-42% with high polyunsaturated fat, to no tumors with low fat (Salim, 1993). Many fermented dairy products containing LAB and probiotic cultures such as *Bifidobacteria* species and *Lactobacillus acidophilus* have been reported to have a positive effect on the immune system. Studies have shown that gastrointestinal cancers induced by DMH could be inhibited by the consumption of products containing LAB. Diets containing a lyophilized culture of *Bifidobacterium longum* were reported to have an inhibitory effect on colon, mammary and liver carcinogenesis (Onoue et al., 1997). The purpose of this study was to determine if LAB in soy and cow milk yogurts had an inhibitory effect against chemically induced colon carcinogenesis in rats.

RESEARCH METHODS

Yogurt preparation

Soy milk and cow milk (Vitamin D-whole milk) were purchased at People's Food Coop., LaCrosse, WI and approximately 1500 gms of yogurt were produced every other week. Yogurt was prepared from milk using commercial yogurt starter cultures (Chr. Hansen, Inc., Milwaukee, WI.) containing *Streptococcus salivarius* sub sp. *thermophilus*, *Lactobacillus delbrueckii* sub. sp. *bulgaricus*, *Bifidobacterium bifidum*, and *Lactobacillus acidophilus*. The milk was measured and granulated sugar was added at 60 g/L. The milk was then autoclaved for approximately one hour and let cool in a water bath. When the temperature of the milk was between 40-43° F, it was inoculated with the bacteria. Both types of milk were inoculated with 0.1 g of each type of bacteria. After 4-5 hours the pH was tested and when the pH was 4.7 or lower the yogurt was deemed finished.

Diet preparation

Powdered diet for rats was purchased from ICN Biochemicals (Costa Mesa, CA) or fresh diet was produced from purified ingredients (ICN Biochemicals, Costa Mesa, CA) according to the AIN 1993 recommendations for rodent growth and maintenance. The caloric content for the powdered diet was 3.18 kcal/g, the soy diet was 2.66 kcal/g and the cow diet was 2.71 kcal/g. The yogurt diets contained 20% yogurt and 80% powdered diet.

1,2-dimethylhydrazine (DMH) preparation and injection

DMH was dissolved in 1 mM EDTA. Sodium hydroxide was added to the solution to bring the pH to 6.5. The rats were injected (20.0 mg DMH or EDTA vehicle solution/kg i.p.) weekly beginning at week 0 and continuing through the end of the study (Onoue et al., 1997).

Animal handling

Twenty-eight, 6-week male Sprague-Dawley rats were housed separately in hanging wire cages with a 12:12 hour light:dark cycle. The rats were divided into 4 groups. The control group of 7 rats was fed only the powdered diet without yogurt and received weekly DMH injections. The reference group contained 7 rats that received the powdered diet and injections of vehicle. The remaining 2 experimental groups contained 7 rats each. They received DMH and were fed the powdered diet mixed with yogurt from different milk sources. Feeding began at week -2 as indicated on the timeline (Figure 1). This allowed variable time for the rats to adjust to the food before injections began. Rats were fed daily based on a 200 kcal/kg/day diet calculated from daily body weight. Food spillage was recorded daily and food intake calculations and body weights were recorded weekly.

Enumeration of bacteria

The viable lactic acid bacteria present in yogurt and fecal samples were enumerated by preparing serial dilutions of the samples in 0.1% (W/V) peptone water. The samples were plated onto *Lactobacillus* MRS agar and incubated under anaerobic conditions at 37° C for 48 hours (Dave and Shah, 1996). Fecal enumeration of total LAB was assessed at -3, +5 and +11 weeks.

Tumor assessment

Following death by carbon dioxide inhalation, the distal 10 cm of the colon was removed and rinsed with phosphate buffered saline. The colons were cut along the longitudinal median axis, fixed flat between filter paper, and let stand in formalin. The colons were later stained with methylene blue (0.2%) for 30-60 minutes so crypt outlines would be visible. The colons were placed on microscope slides, mucosal side up, and examined under a light microscope at 100X total magnification (Bird, 1987). Aberrant crypts were counted based on 2 main criteria: thick borders and increased lumen size of crypts. Crypts were counted by two investigators blind to the condition of the specimens and the counts were averaged. Photographs were taken of the magnified aberrant crypts and the criteria for selection is obvious in Figure 1.

Timeline

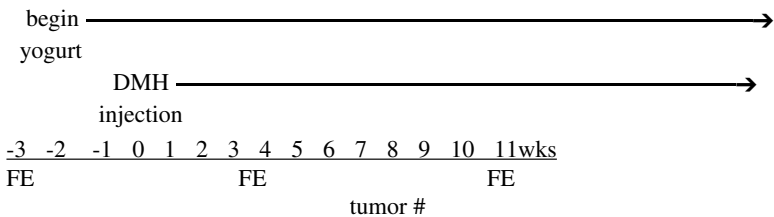


Figure 1: Aberrant crypt tumors in the distal 10 cm of the colon from the DMH-treated rats. Magnification is 290X.

RESULTS

The fecal enumeration data is shown in Figure 2. All 4 groups began the study with approximately 1.00×10^{10} (CFU/g). The three DMH groups exhibited reduced colonization by bacteria up until week +6 relative to the reference group. Both yogurt treated groups exhibited increased colonization until week +6 relative to the control group . Body weights dropped slightly from week -2 to 0, which was expected because of change in diet. From week 0 to week +11 body weights steadily increased in all 4 groups. All data was analyzed using one-way ANOVA and post-hoc t-tests when significance of $p<0.05$ was observed. Body weights at the end of the study were significantly different between the reference and control groups ($p=0.03$) and the reference and soy groups ($p=0.01$) as shown in Figure 3. Food intake increased from week -2 to 0 and then fell slightly which could have been due to the start of DMH injections at week 0 and fluctuated from that point on as shown in Figure 4. Tumor assessment showed a trend with the control group having the highest overall tumor burden (Figure 5). Aberrant crypts were 48.58 ± 10.37 for the control group, 41.21 ± 7.13 for the soy group, 40.96 ± 8.73 for the cow group, and 0 for the reference group. There were no significant ($p<0.05$) differences among DMH-treated groups.

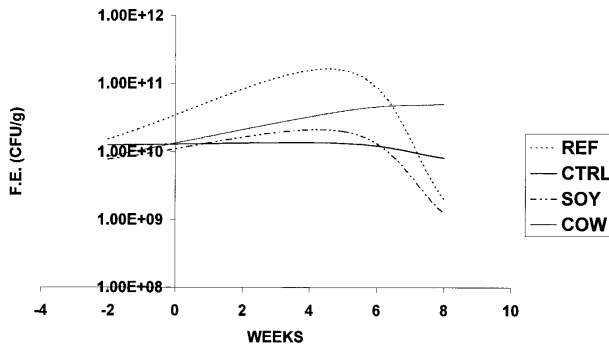


FIGURE 2: Fecal enumeration from DMH-injected rats fed control (CTRL) or soy or cow's milk yogurt diets. REF rats received control diet and no DMH.

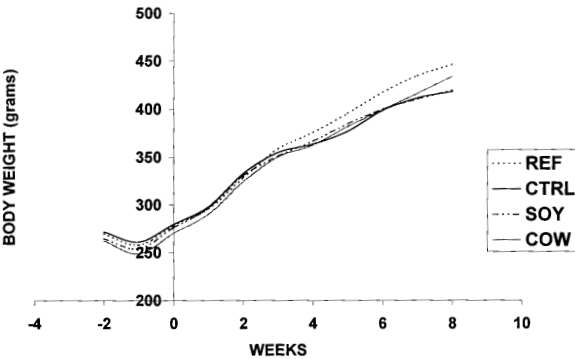


FIGURE 3: Body weight of DMH-injected rats fed control (CTRL) or soy or cow's milk yogurt diets. REF rats received control diet and no DMH.

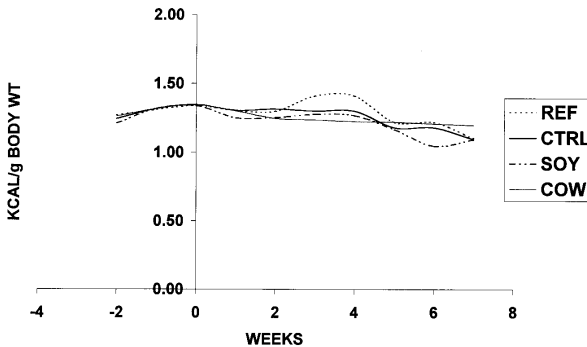


FIGURE 4: Food intake in DMH-injected rats fed control (CTRL) or soy or cow's milk yogurt diets. REF rats received control diet and no DMH.

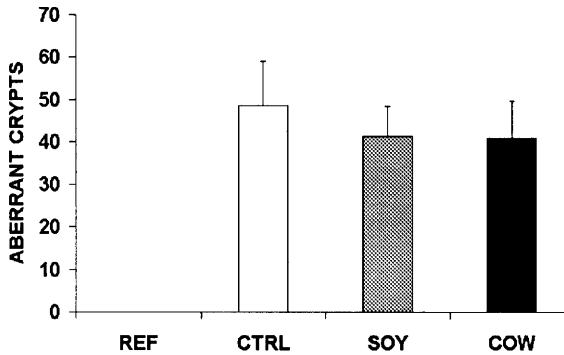


FIGURE 5: Aberrant crypt number in the distal 10 cm of the colon in DMH-injected rats fed control (CTRL) or soy or cow's milk yogurt diets. REF rats received control diet and no DMH.

DISCUSSION

The results from this study show no conclusive anticarcinogenic effect of LAB consumed in yogurt made from cow or soy milk. Addition of yogurt to the rat diets did not significantly alter food intake or growth reflected by body weight. However, DMH did appear to influence growth in all but the cow milk group. Fecal enumeration showed that LAB consumption did increase the colonization of these probiotic bacteria in the intestinal tract, but it appears that DMH reduced bacterial colonization, making the data more difficult to interpret. The tumor burden was significant among all DMH-treated groups reinforcing the reported effects of this colon carcinogen. The dosage of DMH used in this study was on the high end of the literature values and using a lower dosage may increase the sensitivity for detection of the effects of yogurt consumption. Moreover, the antimicrobial effect of DMH, may have reduced any anticarcinogenic effects of LAB that might be apparent with another cancer induction method.

There were many physiological signs observed throughout the study that indicated that the DMH-treated rats were under more stress than the non-DMH-treated rats. In the control, soy, and cow groups chromodactria was noticed around the eyes and nose beginning around week +3. This continued throughout the remainder of the study. The rats in the DMH-treated groups appeared more lethargic the day after injections compared to rats in the reference group. Food intake for the control vs. the soy, cow, or reference group was less for the days after the injections. Another sign of stress that was noticed in the DMH-treated groups was red blotchy patches in the fur. One rat in the control group was euthanized early at week +6 due to self-mutilation of a skin tear. Dissection indicated that the study should be terminated earlier than scheduled. Upon dissection of all rats, the cecums from the DMH-treated groups were swollen significantly in comparison with the cecums from the reference group rats.

In conclusion, although no significant effects of LAB were found, the trend toward a probiotic effect of lactic acid bacteria in preventing colon carcinogenesis merits further study. The functional foods are a rapidly growing area of research and industry that may be of great benefit to human health. Thus, the probiotic effects of LAB consumption should be established in the context of foods versus supplements.

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