Effects of Feeding Sour Milk on Longevity and Tumorigenesis in Mice and Rats

Toshiaki TAKANO,1* Koichiro ARAI,1 Ichiya MUROTA,1 Kunihiko HAYAKAWA,1 Takeo MIZUTANI2 and Tomotari MITSUOKA2,3
Calpis Food Industry Co. Ltd., 2-4-1 Ebisu-Minami, Shibuya-ku, Tokyo 150,1 The Institute of Physical and Chemical Research, Hirasawa, Wako, Saitama 351,2 and Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 1133

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The effects of feeding sour milk on longevity, transplantable tumor, and chemically induced colon tumorigenesis were studied. (1) Female ICR mice were fed with pasteurized sour milk throughout their life. Mice given sour milk had a longer life span than those given the control diet, while the life span of those given whole milk was almost the same as those given the control diet. Necropsy showed that the main causes of death were tumors, renal atrophy, and pneumonia. Sour milk was suggested to inhibit at least one of these diseases. (2) Female ICR mice were intraperitoneally inoculated with Ehrlich ascites tumor cells, and the effect of dietary sour milk on the growth of tumor cells was determined. Feeding pasteurized sour milk or pasteurized starter cells inhibited the growth of tumor cells. The inhibitory effect was dependent on the dose of the starter cells. These results suggested that some component(s) of starter cells was responsible for inhibition of tumor cell proliferation. (3) When male F344 rats were intraperitoneally injected with 1,2-dimethylhydrazine, the effect of dietary sour milk on the incidence of intestinal tumors induced was studied. While the number of colon tumors per animal in rats fed with artificially acidified milk or starter cells was not significantly different from that in rats fed with control diet, rats fed with sour milk developed significantly fewer colon tumors. This finding indicates that sour milk inhibited 1,2-dimethylhydrazine-induced colon tumorigenesis in F344 rats.

Key words: Sour milk; longevity; Ehrlich ascites tumor; 1,2-dimethylhydrazine; colon cancer

Eighty years ago, Metchnikoff claimed in his theory of longevity that ingestion of cultured milk products establishes lactobacillus flora in the intestine, inhibits the growth of putrefactive bacteria, and prevents auto-intoxication of human organs (11). However, subsequent investigators showed that Lactobacillus bulgaricus does not survive in the intestinal tracts. Since that time many efforts have been made to demonstrate the beneficial effects of cultured milk products on health: such as control of blood cholesterol concentration (8), tumor development (15), antibacterial effects (16) and so on. However, the effect of cultured milk products on health and its mechanism have not been made completely clear.

In our serial studies, the effects of a type of cultured milk product on longevity (1) and tumors, transplantable tumor (2) and chemically-induced colon tumorigenesis were demonstrated by using mice and rats.

1. Effect of Sour Milk on Longevity

Three groups of female ICR mice were fed
with either the diet containing 14% sour milk, or the diet containing 1.6% whole milk powder or the control diet throughout their life from 4 weeks of age. The sour milk was prepared as follows: pasteurized skim milk was inoculated with starter culture which contained Lactobacillus helveticus ss. jugurti LB and Candida utilis A6, fermented at 37°C for 24 hr in industrial scale and pasteurized.

Nutritional analysis showed that the compositions of all the diets were almost the same (Table 1). Figure 1 shows the changes in the survival ratio throughout the experimental period. The average life span of the control group, whole milk group and sour milk group was 84.9, 84.4 and 91.8 weeks, respectively. The life span of the sour milk group was, on the average, 8% longer than that of the control group, while the average life span of the whole milk group was the same as that of the control group. This finding indicated that feeding sour milk prolonged the life span of mice. However, little difference in maximum life span among the 3 groups was observed (Fig. 1). The prolongation of the average life span was considered to be a result of the preventive effect of sour milk on death caused by some disease. As shown in Table 2, the main causes of death were renal atrophy, tumors, and pneumonia, though there were few differences in the analysis of the deaths among the groups. These findings suggested that the sour milk inhibited at least one of these diseases but not some specific disease.

Changes in the intestinal microflora during the experimental period is shown in Fig. 2. The number of enterobacteriaceae, streptococci and lactobacilli did not significantly differ among the 3 groups. However, the counts of bifidobacteria in the intestinal microflora of mice fed with sour milk were 10 times higher than those fed with either whole milk or the control diet. The effect of sour milk on the life span of mice might be
2. Effect of Sour Milk on Ehrlich Ascites Tumor

The effect of sour milk on tumor, one of the main diseases causing the death of mice in the experiment on life span, was studied. Female ICR mice of 6 weeks of age were intraperitoneally inoculated with $1 \times 10^6$ cells of Ehrlich ascites tumor cells. Mice were given the sour milk mixed with the control diet or with drinking water. The ascites tumor growth in mice was determined at the sacrifice of them.

Sour milk and yoghurt: The sour milk prepared as described above or yoghurt fermented by *L. bulgaricus* and *Streptococcus thermophilus* were mixed with water and fed to the mice. The development of tumor cells in the mice given sour milk was slower than that in those given the control diet (Fig. 3). Both the pasteurized and non-pasteurized yoghurt inhibited the growth of tumor cells by 28% of the control, whereas the sour milk inhibited the growth by 42% of the control.

Lactic acid and skim milk: To investigate what kind of component(s) of sour milk is responsible for the inhibition, mice were fed with skim milk, sour milk, or lactic acid which is the main product of sour milk fermentation. The growth of tumor cells was inhibited by sour milk, but not by lactic acid or skim milk (Fig. 4). This finding suggested that some fermentation product(s) other than lactic acid, including starter cells, were effective in inhibition of the growth of tumor cells.

Starter cells and culture supernatant: *L. helveticus* ss. *jugurti* LB was cultured in Briggs liver broth and *C. utilis* A6 was cultured in potato dextrose broth. These cells were separated by centrifugation. Mice were fed with the supernatant or the cells (which were
Fig. 3. Effect of administration of yoghurt or sour milk to mice on the proliferation of Ehrlich ascites tumor cells. •: Control, ○: yoghurt (pasteurized), △: yoghurt (not pasteurized), □: sour milk (pasteurized).

Fig. 4. Effect of administration of skim milk, lactic acid or sour milk to mice on the proliferation of Ehrlich ascites tumor cells. •: Control, ○: skim milk, △: lactic acid, □: sour milk.

Fig. 5. Effect of administration of the cells of *L. helveticus ss. jugurti* LB or the culture supernatant to mice on the proliferation of Ehrlich ascites tumor cells. •: Control, △: supernatant (not pasteurized), ▲: supernatant (pasteurized), □: cells (not pasteurized), ■: cells (pasteurized).

Fig. 6. Effect of number of cells of *L. helveticus ss. jugurti* LB administered to mice on the proliferation of Ehrlich ascites tumor cells. •: Control, ○: 10⁶ cells/ml, △: 10⁷ cells/ml, □: 10⁸ cells/ml, ▼: 10⁹ cells/ml.
resuspended in distilled water) from each microorganism either pasteurized or not. Whereas the cells of L. helveticus, both pasteurized and not, inhibited the growth of tumor cells, culture supernatant did not (Fig. 5). In C. utilis, the result was the same (data not shown).

**Dose of starter cells:** Mice were fed with the cells of L. helveticus or C. utilis at different concentrations. Remarkable inhibition of the growth of tumor cells was observed in mice fed with a large amount of cells, but not in those fed with a small amount of cells (Figs. 6 and 7).

It has been known that peptidoglycan of bacterial cell walls has adjuvant activity. Anti-tumor glycopeptide is isolated from the cell wall of L. bulgaricus used for yoghurt production (3). These glycopeptides are also active as an adjuvant when orally administered (12). Feeding mice with yoghurt inhibits the growth of transplantable tumor cells (15) and some component of starter cells have the anti-tumor activity (5). The inhibitory effect of sour milk on Ehrlich ascites tumor cells might be elucidated by such antitumor activity of starter cell component through the host immune system.

### 3. Effect of Sour Milk on Chemically-induced Colon Tumorigenesis

Epidemiologic studies suggest that dietary factors, particularly high intake of meat or fat and relative lack of dietary fiber, may be important in etiology of large bowel cancer (4, 17). Animal model studies also demonstrated that colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) is promoted by high intake of meat or fat (13), and inhibited by dietary fiber (10). Goldin and Gorbach (7) demonstrated that feeding viable L. acidophilus inhibits the DMH-induced colon carcinogenesis in rats. However, as yet, the effect of cultured milk products on chemically-induced colon carcinogenesis has never been reported in the literature.

Male F344 rats of 6 weeks of age were fed with one of 4 test diets: sour milk, artificially acidified milk, starter cells, and control commercial diet (CE-2, Nippon Clea). Sour milk was mixed with the control diet in 28%. Artificially acidified milk was prepared by adding 2.4% DL-lactic acid to skim milk. Starter cells, $1 \times 10^9$/ml L. helveticus and $1 \times 10^6$/ml C. utilis, were prepared as mentioned above. Both the artificially acidified milk and the starter cells were mixed with the control diet in the same proportion as the sour milk. From 6 weeks of age 9 rats in each group received a weekly intraperitoneal injection of DMH solution (20 mg/kg body weight) 16 times, and fed with the test diets for 10 weeks after the last injection. At 32 weeks of age, all rats were examined grossly and histopathologically for incidence of tumor.

The incidence of colon tumors is shown in Table 3. Animals with colon tumors were slightly fewer in the sour milk group than in the control group, although statistically not significant. The average number of colon tumors per animal was significantly lower ($p<0.05$) in the sour milk group than
Table 3. Incidence of DMH-induced colon tumors in rats fed a diet containing sour milk, artificially acidified milk, or starter cells

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Animal with colon tumors</th>
<th>No. of colon tumors per rat</th>
<th>Animals with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>9</td>
<td>2.6</td>
<td>6</td>
</tr>
<tr>
<td>Sour milk</td>
<td>9</td>
<td>6</td>
<td>1.0a</td>
<td>0</td>
</tr>
<tr>
<td>Acidified milk</td>
<td>9</td>
<td>9</td>
<td>3.4</td>
<td>7</td>
</tr>
<tr>
<td>Starter cells</td>
<td>8</td>
<td>7</td>
<td>2.3</td>
<td>7</td>
</tr>
</tbody>
</table>

* Significantly different from control p<0.05.

Table 4. Incidence of small-intestinal tumor in rats fed a diet containing sour milk, artificially acidified milk, or starter cells and treated with DMH

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Animal with small-intestinal tumors</th>
<th>No. of small-intestinal tumors per rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>4</td>
<td>0.4</td>
</tr>
<tr>
<td>Sour milk</td>
<td>9</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>Acidified milk</td>
<td>9</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>Starter cells</td>
<td>8</td>
<td>7</td>
<td>1.4</td>
</tr>
</tbody>
</table>

in the control group by Poisson analysis. This finding showed that sour milk inhibited DMH-induced colon tumorigenesis in F344 rats. However, the artificially acidified milk group developed almost the same number of colon tumors per animal as the control. In the starter cell group the number of colon adenocarcinoma was fewer than that in the control group or acidified milk group, whereas the number of colon adenoma or total tumors in the same group did not markedly differ from that of the control group.

The number of small-intestinal tumors did not differ markedly among the sour milk, acidified milk, and control group, whereas that of the starter cell group was higher than those of the other three groups (Table 4).

The inhibitory effect of sour milk on Ehrlich ascites tumor may be explained as the anti-tumor effect of the starter cell component through the host immune system. However, the inhibitory effect of sour milk on the DMH-induced colon tumorigenesis is unable to be sufficiently explained as the effect of starter cell component because the colon tumorigenesis was not inhibited by the starter cells.

Intestinal microflora is postulated to play an important role in colon carcinogenesis (14). Mizutani and Mitsuoka (9) demonstrated that the presence of *Bifidobacterium longum* in the intestine reduces the incidence of liver tumor by other intestinal organism in gnotobiotic C3H male mice. Certain enzymes of intestinal microflora, such as β-glucuronidase, nitroreductase, azoreductase etc. play an important role in the metabolism of carcinogens. Goldin and Gorbach (6, 7) reported that feeding *L. acidophilus* reduced the activity of these enzymes and inhibits DMH-induced colon tumors in rats consuming a beef diet. In the experiment on life span of mice mentioned above, it was observed that feeding the sour milk increased the number of fecal bifidobacteria. This effect of the sour milk on intestinal microflora might play a role in inhibition of colon tumorigenesis.

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References


