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SHORT REPORT

Commercial cows' milk has uterotrophic activity on the uteri of young ovariectomized rats and immature rats

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Cows' milk contains considerable quantities of estrogens, mainly in the form of estrone sulfate (ES). To determine whether the commercial milk has any biologically significant hormonal effects, 2 series of uterotrophic tests were performed, 1 with young ovariectomized rats and the other with sexually immature rats. Thirty-six rats were used for each test. They were divided into 3 groups of 12 animals each, and were kept for 7 days on powdered chow with 1 of 3 drinking solutions: low-fat milk (LFM), artificial milk (AM, negative control), or AM containing ES at 100 ng/ml (positive control). At autopsy, both the wet and blotted uterine weights were measured. The cell heights of uterine epithelia in ovariectomized rats were also determined. The significance of differences among groups was tested by Dunnett's multiple comparisons test. In each test, the weights of the uteri in the LFM group were significantly greater than those of the respective weights in the AM group ($p < 0.01$). Furthermore, in ovariectomized rats, the uterine epithelial-cell height in the LFM group was significantly greater than that observed in the AM group ($p < 0.01$). The uterotrophic effect of 100 ng/ml ES solution was greater than that of LFM in immature rats ($p < 0.01$), whereas the effect of the solution was almost comparable to that of LFM in young ovariectomized rats ($p > 0.05$). In conclusion, commercially available milk has uterotrophic effects in both young ovariectomized rats and sexually immature rats.

Key words: cows'milk; positive uterotrophic test; ovariectomized rats; immature rats

Cows' milk contains considerable amounts of estrogens (estrone, estradiol-17β and estriol).[1] Because of modern breeding practices, 75% of commercial milk comes from cows during pregnancy, when the estrogen levels in their blood, and hence in their milk, are elevated.[2] The hormone levels in milk exceed those in blood, probably owing to hormone synthesis in the mammary glands.[3]

The major estrogen in milk is estrone sulfate (ES),[4] which when consumed can be readily converted into estrone or estradiol-17β.[5] Because of its hydrophilic nature, this main conjugate can be easily absorbed from the gastrointestinal tract. Quantitatively, ES is the most important blood estrogen.[6,7] Exogenously administered ES has been shown to stimulate mammary tumor growth.[8]

To determine whether the cows' milk on the market has any biologically significant hormonal effects, 2 series of uterotrophic assays were performed, 1 with ovariectomized young rats and the other with sexually immature female rats.

Material and methods

The low-fat (1%) milk used in this study (Holstein milk sterilized at 130°C for 2 sec) was the same one as that was used previously.[9] The artificial milk (AM), which was used as a negative control solution, contained the same amount of protein (gluten fortified with lysine, DL-methionine, threonine and valine), fat (coconut oil) and carbohydrate (dextrin maltose) as the low-fat milk. The composition of the AM has been described elsewhere.[9] A solution of ES in the AM (100 ng/ml) was used as a positive control solution. The sulfated estrone (3-hydroxyestra-1,3,5(10)trien-17-one) was obtained from Sigma Chemical Company (Tokyo, Japan).

The care and use of laboratory animals followed the Guidelines for Animal Experiments of the Medical University of Yamanashi.

Ovariectomized rats

Female Wistar Galas Hannover rats, ovariectomized at 6 weeks, were purchased from Nippon Clea (Tokyo, Japan). Upon receipt, the rats were housed, 3 per polycarbonate cage, inside an air-conditioned animal room (22°C ± 2°C) with artificial lighting from 06:00 to 18:00 hr; the rats were provided with a diet of powdered chow (CE-2, Nippon Clea) and water. After a week of acclimatization, the rats at 8 weeks of age were weighed, numbered and randomly assigned to 3 groups of 12 animals each. Each group was then maintained on the powdered chow plus 1 of the following 3 test solutions as the only drinking fluid: low-fat milk (LFM); AM (negative control); or AM-containing ES (positive control). Food and liquid solutions were renewed daily at 10:00 hr. Daily consumption was determined as the difference between that which was provided and that which remained unconsumed at 10:00 hr the next day. The consumption was recorded in grams per cage (3 rats) per day. Body weight was measured every day, starting just prior to the change of dietary regimen.

Immature rats

Thirteen-day-old immature female Wistar Galas Hannover rats were obtained from Nippon Clea (Tokyo, Japan) as litters accompanied by the dam or a foster dam. Upon receipt, the rats were housed, 1 litter per polycarbonate cage, in the same air-conditioned animal room described above and were provided with powdered chow and water. When the baby animals reached 17 days of age, they were weighed, numbered and randomly assigned to 1 of the 3 groups (LFM, AM or ES), which each consisted of 12 rats. The immature female rats were then treated essentially as described above for the ovariectomized rats, excepting that the vaginal opening in immature rats was checked daily.

Autopsy

After being maintained on the test liquids for 7 days, the animals were killed by ether inhalation at 16:00 hr, ~24 hr after the last treatment. The uteri were dissected free from adhering fats, and the wet uterine weights were recorded to the nearest 0.1 mg. Then, the tip of each uterus was cut, and the uterus was placed on filter paper and gently pressed to blot the fluid. The blotted weights of the uteri were recorded. The cell heights of uterine epithelia in ovariectomized rats were determined on HE-stained sections using a microscope (Olympus BX 50, Tokyo, Japan) with an attached image analyzer (Nihon Digital, Tokyo, Japan), according to Newbold *et al.*[10]

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TABLE I – EFFECTS OF LOW-FAT MILK ON THE UTERINE WEIGHTS OF OVARIECTOMIZED RATS

	Body weight (g, A)	Wet uterine weight (mg, B)	Blotted uterine weight (mg)	B/*A*
Artificial milk (AM)	228.4 ± 9.2	60.2 ± 6.1	45.5 ± 5.9	0.265 ± 0.030
Low-fat milk (LFM)	227.9 ± 7.8	72.6 ± 7.2[1]	53.9 ± 6.1[1]	0.319 ± 0.030[1]
Estrone sulfate in AM (ES)	229.6 ± 11.6	76.0 ± 5.0[1]	56.8 ± 6.8[1]	0.332 ± 0.026[1]

Values given are mean ± SD.
[1]Significantly different from AM (negative control) ($p < 0.01$).

TABLE II – EFFECTS OF LOW-FAT MILK ON THE UTERINE WEIGHTS OF IMMATURE RATS

	Body weight (g, A)	Wet uterine weight (mg, B)	Blotted uterine weight (mg)	B/*A*
Artificial milk (AM)	37.9 ± 4.0	24.2 ± 3.0	21.7 ± 3.2	0.643 ± 0.099
Low-fat milk (LFM)	38.6 ± 3.3	36.7 ± 3.8[1]	33.3 ± 4.3[1]	0.959 ± 0.149[2]
Estrone sulfate in AM (ES)	39.2 ± 6.0	71.3 ± 14.6[1,3]	68.8 ± 14.0[1,3]	1.850 ± 0.456[1,3]

Values given are mean ± SD.
[1]Significantly different from AM (negative control) ($p < 0.01$);–[2]$p < 0.05$.–[3]Significantly different between LFM and ES (positive control) ($p < 0.01$).

Statistical analysis

All data were analyzed by ANOVA using SPSS (SPSS Inc., Chicago, IL), followed by Dunnett's multiple comparisons test, when significant differences existed among groups. The 0.05 level of probability was used as the criterion of significance.

Results

Ovariectomized rats

Body weights were comparable among the 3 groups (Table 1). Both wet and blotted weights of the uteri in the LFM group were significantly greater than the respective weights in the AM group (negative control) ($p < 0.01$). The ratio of the wet uterine weight to the body weight was also significantly higher in the LFM group than in the AM group ($p < 0.01$).

The wet and blotted weights of the uteri in the ES group (positive control) were also significantly greater than the respective weights in the AM group ($p < 0.01$). The ratio of the uterine weight to the body weight was higher in the ES than that in the AM group ($p < 0.01$) (Table 1). In general, both the absolute and relative values of the uterine weights were higher in the ES group than those in the LFM group, but the difference between the 2 groups was not statistically significant ($p > 0.05$).

The thickness of the uterine epithelia (mean ± SD in μm) was significantly greater in the LFM group (8.4 ± 1.4) than that in the AM group (6.8 ± 1.6) ($p < 0.01$). The uterine epithelial cells of rats in the ES group (9.4 ± 1.7) were significantly higher than the cells of rats in the AM group ($p < 0.01$). The difference in the cell heights between the LFM and ES groups was not statistically significant ($p > 0.05$).

Immature rats

No significant difference in body weight was noted among the 3 groups (LFM, AM and ES) of immature rats (Table 2). Both wet and blotted uterine weights of rats in the LFM group were significantly greater than those of rats in the AM group ($p < 0.01$), respectively. The ratio of the wet uterine weight to body weight was also significantly higher in the LFM group than that in the AM group of immature rats ($p < 0.05$).

All the uterine values of rats in the ES group were significantly higher than the respective values of rats in both the AM and the LFM groups ($p < 0.01$).

None of the immature animals used had an open vagina during the uterotrophic assay (18–24 days-old).

Discussion

There is growing concern regarding the decline of reproductive health,[11–14] the increased incidence of hormone-dependent cancers[15–20] and the frequent occurrence of premature thelarche.[21] Although endocrine-disrupting agents in the environment were blamed for these phenomena,[22] the possible role of endogenous estrogens from food has not been widely discussed. Nevertheless, the relative potency of estradiol-17β is 10-fold to 100,000-fold that of most identified xenoestrogens.[23]

The uterotrophic assay is considered the ''gold standard'' and is an essential component when testing for estrogenicity, as it incorporates the effects of metabolism and pharmacokinetics.[24] The present study clearly indicates that commercially available LFM has a weak but significant uterotrophic effect on both young ovariectomized rats and immature rats with intact, undeveloped uteri.

The LFM used in the present study contained about 380 pg/ml ES, in addition to 210 pg/ml estradiol-17β (free + conjugated) and 50 pg/ml estriol (free + conjugated).[9] The uterotrophic effect of the milk was similar to the effect of 100 ng/ml ES in the ovariectomeized rats (Table 1). However, the effect of ES at the same concentration was much more pronounced in the immature rats than that in the young matured rats (Table 1 *vs*. Table 2). The uteri of immature rats may be more sensitive than are the uteri of young but sexually matured rats.[25]

None of the immature animals had an open vagina during the uterotrophic assay. Nonetheless, milk and ES produced a clear uterotrophic effect. The observation of premature vaginal opening appears to be a less sensitive marker of estrogenic activity than is the stimulation of uterine growth, as has been previously reported.[26]

In conclusion, commercially available milk has uterotrophic effects in both young ovariectomized rats and sexually immature rats. Further studies are necessary to ensure the safety of milk and dairy products, particularly concerning their hormonal effects.

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