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 uterotropic 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). 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. The hormone levels in milk exceed those in blood, probably owing to hormone synthesis in the mammary glands. The major estrogen in milk is estrone sulfate (ES), which when consumed can be readily converted into estrone or estradiol-17β. Because of its hydrophilic nature, this main conjugate can be easily absorbed from the gastrointestinal tract. Quantitatively, ES is the most important blood estrogen. Exogenously administered ES has been shown to stimulate mammary tumor growth. 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 used previously. 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. 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, in the same 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 uterus 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 the microscopic stage of a microscope (Olympus BX 50, Tokyo, Japan) with an attached image analyzer (Nihon Digital, Tokyo, Japan), according to Newbold et al.