The effect of dietary xylitol on dentin formation in ovariectomized rats

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Twenty-nine 3-month-old female Wistar rats were labeled by means of a single intraperitoneal tetracycline injection. Nineteen animals were subsequently ovariectomized, whereas a control group of 10 animals underwent sham operations. All the animals received the basal diet, and 10 of the ovariectomized animals were given an additional dietary xylitol supplementation (5%). Three months later the animals were killed by decapitation, and dentinal apposition on the molars was measured. The results indicate that supplementation of the diet with 5% xylitol had an attenuating effect on the enhanced dentin formation caused by ovariectomy, but the mechanism remains unsolved. □ *Dentin apposition; osteoporosis; xylitol*

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Ovariectomy, which is used as a model for type-I osteoporosis (1), is known to enhance dentinal apposition (2) and periosteal bone formation in adult rats (3, 4). It has previously been postulated that odontoblasts and osteoblasts have functional similarities in common (5), and the findings concerning ovariectomy further support this and suggest a relationship between odontoblastic and osteoblastic responses.

Xylitol has been shown to increase calcium absorption in rats (6, 7), possibly by calcium–xylitol complex formation (8, 9), and an activation of the paracellular route is suggested (10). A dietary xylitol-induced promotion of calcium and phosphorus concentration (11) and accelerated calcification in previously calcium-deficient bone have been observed in rats (12), and there is some evidence of reduced dentin apposition in connection with dietary xylitol (13).

The present study was carried out to determine whether xylitol can normalize the enhanced dentin formation caused by ovariectomy.

Materials and methods

A total of 29 Wistar rats were weaned at 22 days of age and fed ad libitum on a basal pellet diet (Ewos R3, Södertälje, Sweden) and distilled water to the age of 16 weeks. At the age of 15 weeks 19 of them were ovariectomized by the dorsal approach under fluanisone-fentanyl (Hypnorm®, Janssen, Beerse, Belgium; 1:1 sterilized water) and midazolam (Dormicum®, Roche, Basel, Switzerland; 1:1 sterilized water) anaesthesia (1:1, 0.2 ml/100 g), and 10 were sham-operated. Oxytetracycline (Terramycin®, 100 mg/ml (as hydrochloride), Pfizer, Brussels, Belgium) was injected intraperitoneally after the operation to mark the mineralizing dentin front.

After recovery for 1 week, a dietary regimen was started in which 9 of the ovariectomized rats were maintained on a powdered basal pellet diet and distilled water ad libitum (O+) and 10 on the same diet with 5% xylitol added (Cultor Co., Espoo, Finland) (OX). Ten sham-operated rats were used as controls, on the same diet as group O+ (cnt).

Having been housed in groups of 10 in large cages (Makrolon IV) before the experiment, the rats were moved for the experiment to smaller cages (Makrolon III), 2 or 3 to a cage, on a bed of European aspen shavings with a 12/12-h light schedule, a

room temperature of 21°C and 40–60% humidity. They were decapitated under ether anesthesia after 3 months, at which point the success of ovariectomy was verified by failure to detect any ovarian tissue and marked atrophy of the tuba uterina and related arteries. The lower jaws were prepared, hemisected sagittally in the mesiodistal plane as described by Keyes (14), and one main fissure from each mandibular molar photographed with Kodak Ektachrome daylight film (400 ASA) under a microscope, using ×16 magnification (Orthoplan Ploemopak, Leitz, Westlar, Germany), ultraviolet light (mercury vapor lamp C2, 200W/4, Philips, Belgium), and a 460-nm detector to reveal the tetracycline fluorescence. Dentinal apposition during the experiment was determined by outlining the areas encircled by the tetracycline fluorescence under the fissures as they appeared on the monitor (Camera Hitachi VK 98 E, monitor Salora 445 A RGB) via a serial mouse connected to a PC Vision Frame Grabber (Imaging Technology, Woburn, Mass., USA) and an Image Measure computer program (Microscience, Washington, DC, USA).

Statistical differences between the experimental groups were assessed with ANOVA together with Tukey's standardized range test ($p < 0.05$).

Results

The weight gains of the ovariectomized animals were markedly greater than those of the controls during the 3 months ($p < 0.05$). During the experimental period the gain was 113.1 ± 15.6 g in the ovariectomy group, 100.9 ± 13.4 g in the ovariectomy with dietary xylitol, and 54.5 ± 17.2 g in the sham-operated control group.

Ovariectomy caused an up to twofold increase in dentinal apposition compared with sham-operated rats, the difference being statistically significant in all the molars ($p < 0.05$) (Fig. 1). This effect was normalized by xylitol. The difference in dentinal apposition between the ovariectomized xylitol rats and the controls was not statistically

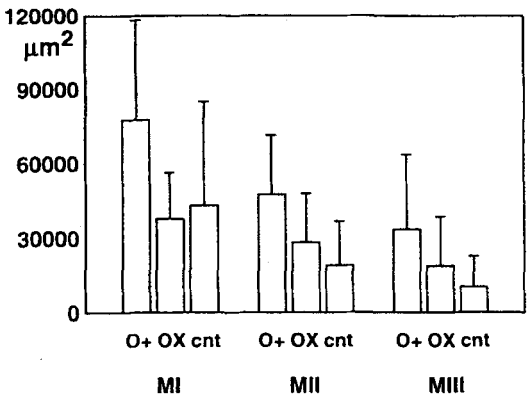

Fig. 1. Areas of molar dentin formation during the experiment (+SD). The following statistically significant differences were found using ANOVA with Tukey's test ($p < 0.05$): MI, O+ versus cnt, OX versus cnt; MII, O+ versus cnt, OX versus cnt; and MIII, O+ versus cnt. Abbreviations: O + = ovariectomized rats; OX = ovariectomized rats with dietary xylitol supplementation; cnt = sham-operated controls; MI = first molar; MII = second molar; MIII = third molar.

significant, but that between the ovariectomized xylitol rats and ovariectomized rats fed a normal diet was statistically significant ($p < 0.05$) in all the molars except MIII (Fig. 1). The results concerning dentinal apposition were based on the method of Keyes (14), and the measurements were performed in one plane of the sagittally hemisected molars (Fig. 2a and b). The above procedure had earlier been proved to be a valid and reliable method for measuring dentinal apposition (15).

Discussion

The finding of enhanced dentinal apposition in the ovariectomized rats is in line with previous observations (2), and its normalization by dietary xylitol supplementation is parallel with the earlier demonstrated xylitol-caused prevention of bone mineral loss after ovariectomy (16). The mechanism of these effects of xylitol, however, remains obscure.

The involvement of a reduced vitamin D concentration during dietary xylitol sup-

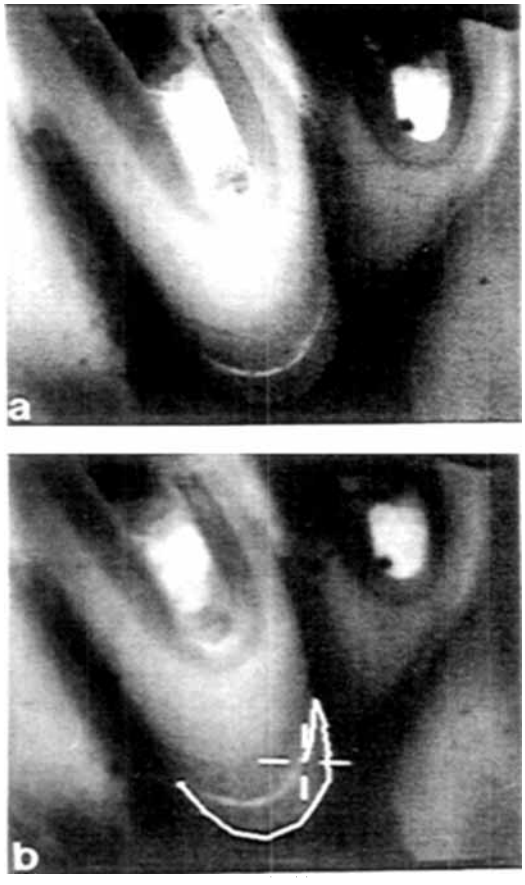

Fig. 2a. A sagittally hemisected rat second molar showing the tetracycline injected at the beginning of the experiment, viewed under a microscope equipped with ultraviolet light. 2b. Video image of the dentinal apposition formed during the experimental period, encircled with a serial mouse connected to a PC Vision Frame Grabber and image measurement computer program.

plementation, found by Hämäläinen et al. (6), may be one factor explaining the present findings. Vitamin D deficiency is thought to cause a widening of the predentin, indicating a delay in calcification (17). Messer & Guo (18) found that for any given level of dietary calcium, the rate of dentin mineralization in vitamin D-deficient rats was approximately half of that in vitamin D-supplemented ones, whereas Ferguson & Hartles (19) found that when the diet lacked vitamin D, the mass of tooth formed was reduced owing to a deficiency in calcium. Even though the predominant effect of vitamin D is not on the odontoblasts, there is evidence that it does have some effect on the supporting tissues such as the pulp cells (20). The possible effect on dentin may be mediated in this manner, as pulpal cells (21), possibly fibroblasts (22), have been shown to participate in the formation of new dentin.

Contrary to others, Engström et al. (23) reported increased protein synthesis by odontoblasts in vitro and increased odontoblast metabolism in vitamin D-deficient rats (24, 25). This speculation leads us to the notion that enhanced osteoblast and odontoblast functions are not mediated via vitamin D action in estrogen deficiency osteoporosis but by other enhancing factors. This is supported by the findings of Turner et al. (26) that ovariectomy did not alter mean serum 1, 25 $(OH)_2D_3$ levels in *growing* rats, but the bone apposition rate was increased. There is no evidence of reduced vitamin D content either after ovariectomy per se or connected with xylitol supplementation in adult rats.

On the other hand, xylitol has metabolic effects that may also have implications for dentin formation. These include some similarities with ethanol metabolism, such as an increase in the ratio of nicotinamide adenine dinucleotide, reduced form, to nicotinamide adenine dinucleotide (27, 28) and a reduction of vitamin D concentrations (6, 29), both of which have been shown to influence bone mineralization (30, 31). Besides, ethanol has been reported to reduce collagen synthesis in fibroblasts. As secondary dentin is thought to be synthesized by pulpal fibroblasts (22), the above metabolic changes could also partly explain the effect of xylitol on dentin formation.

In conclusion, xylitol may be regarded as attenuating increased dentinal apposition caused by ovariectomy.

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