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Effect of Silicon Supplement on Osteopenia Induced by Ovariectomy in Rats

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Abstract. The effect of silicon (Si) supplement on preventing bone mass loss induced by ovariectomy (OVX) in rats was investigated. Three groups of 15, 100-day-old female Wistar rats each, with a mean initial weight of ~260 g per animal, were selected for the present study. One of the experimental group consisting of 15 OVX rats was fed a diet supplemented with 500 mg of Si per kg of feed (Si + OVX). The other two groups consisting of 15 OVX and 15 sham-OVX rats did not receive these supplements. Morphometric (weight and length) and densitometric studies with dual-energy X-ray absorptiometry were performed on the whole femur and 5th lumbar vertebra of each animal 30 days after the experiment. The Si + OVX rats did not show a loss of bone mass induced by OVX at axial level (5th lumbar vertebra) or periphery (femur). Nonetheless, a significant increase (ANOVA with Bonferroni/Dunn post hocs test) of longitudinal development of the femur ($P < 0.0001$) was patent. These results, obtained through the measurements of axial and peripheral bones, warrant closer scrutiny in connection with the Si inhibitory effect on bone mass loss as well as the stimulatory effect on bone formation. Both actions, namely, inhibition of resorption and stimulation of formation, infer that Si may have a potential therapeutic application in the treatment of involutive osteoporosis.

Key words: Silicon — Ovariectomy — Bone loss — Rats — Bone mineral content — Bone mineral density.

Silicon (Si) is an essential mineral in the animal diet [1] to such an extent that it is preferentially found in the growth regions of the body and consequently it has been considered an important element that plays a crucial role in bone calcification [2]. In addition to bone, Si deficiency is manifested by abnormalities involving articular cartilage and connective tissue. Chicks from the Si-deficient group had thinner legs and smaller combs in proportion to body size. Likewise, their long-bone tibial joints were markedly smaller and bones contained 34–35% less water than the chicks whose diet was supplemented with Si [3].

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Similar to the examples above, supplements of 50 mg Si/100 g of diet stimulates rat growth and increases body weight [4]. Carlisle [5] observed that by supplementing chick diets with Si, as it was done by Schwartz and Milne [4], the body weight increased significantly in less than a month. Both experiments have proven beyond a doubt that the effect of Si favors somatic development in experimental animals. In 1983, Parfitt [6], while referring to bone and the risk of excessive bone mass loss synonymous with osteoporosis, stated that several trace elements including zinc and Si were essential for normal bone growth and development. In this sense, Si seems to be indispensable for the synthesis of bone marrow, given its osteoformatory effect on the collagen or bone matrix [7].

Experimental rat models are considered to be suitable for extrapolation of results to human subjects [8, 9]. The usefulness of dual-energy X-ray absorptiometry (DXA) for bone-mass determinations in small animals has been validated repeatedly [10] and our team had relied extensively on this technique in previous experimental studies [11, 12]. More recently [13], we have demonstrated that zinc exerts a powerful influence by inhibiting the loss of bone mass in rats subjected to strenuous physical exercise. In view of these assertions, the present study was centered on the verification of the effect of Si on the bone mass loss through ovariectomy practiced on rats.

Material and Methods

Three batches (15 animals each) of 100-day-old female Wistar rats with a mean initial weight of ~260 g were investigated. All rats were fed Mucedola type 4RF21 (Mucedola s.r.l. Milano, Italy) feed containing 7.1 g/kg calcium and 5 g/kg phosphorus; the energy content of the feed was 3100 kcal/kg. The rats were kept for 30 days in the animal laboratory of the University of Alcalá, Madrid (Spain). Living conditions (12 hours of light and 12 hours of dark cycles), mean room temperature (22°C), habitat, and the diet were observed in accordance with current guidelines imposed by the European Union Council. Experimental procedures were followed according to the guiding principles on Care and Use of Animals as approved and overseen by the appointed institutional animal care committee. Sample size was calculated in a pilot study after determining the variability of densitometric measurements. The standard deviation (SD) of bone mineral density (BMD) was 10 and the hypothesized difference among the groups was 15 units.

Table 1. Group characteristics of three groups of rats

	OVX	Sham-OVX	Si + OVX
n	15	15	15
Initial weight (g)	257 ± 14	258 ± 17	269 ± 21
Final weight (g)	321 ± 20	293 ± 16[a]	363 ± 36
Femur length (mm)	33.4 ± 0.7[a]	34.1 ± 0.8	34.2 ± 0.7
Femur (mg)	649 ± 44[a]	701 ± 46	738 ± 62
F-BMC (mg)	295 ± 31[a]	344 ± 13	375 ± 30[b]
F-BMC/FW (mg/g)	0.92 ± 0.07[a]	1.18 ± 0.07	1.04 ± 0.08
F-BMD (mg/cm^2)	0.11 ± 0.010[a]	0.12 ± 0.008	0.12 ± 0.006
Vertebra (mm)	6.2 ± 0.6	6.1 ± 0.4	6.2 ± 0.5
Vertebra (mg)	207 ± 25[a]	241 ± 20	251 ± 22
V-BMC (mg)	79 ± 16[a]	113 ± 13	106 ± 15
V-BMD (mg/cm^2)	101 ± 9[a]	112 ± 8	111 ± 8

[a] $P < 0.0001$ vs others
[b] $P < 0.005$ vs sham-OVX according to ANOVA with Bonferroni/Dunn post hocs test

Alpha risk was found to be 0.05 and beta risk was 0.20 for a two-sided control. The number of animals in each group was established according to the formula nc = ne = 2(Za + Zb)2s2/D2, where nc = number of animals in the control group; ne = number of animals in the experimental group; s = SD; D = difference to be detected.

The rats were randomly integrated into three groups based on their body weight. Those with highest and lowest weight were alternatively incorporated into one of the groups, so that at the end of the randomization the mean body weight of each group was easily comparable. All of the 45 animals were anesthetized intraperitoneally with ketamine hydrochloride (10 mg/kg) and acepromazine (3 mg/kg), their abdomens were shaved, and the skin was cleaned with 70% ethanol and povidoneidone (Betadine) solution. A longitudinal midline incision was made in the subumbilical region to expose the rectus abdominous muscle and the abdominal cavity. The urinary bladder was retracted and the uterine horns were identified. The ovarian arteries were ligated and bilateral ovariectomy (OVX) was performed in 30 animals; in the remaining 15 rats the ovaries were exposed but not excised (sham-OVX). In all the other animals the abdominal musculature was sutured and the skin was closed with staples.

The experimental groups of OVX rats were fed the common diet supplemented with an additional 50 mg Si/100 g of diet (500 ppm) as sodium metasilicate (Na2SiO3-9H2O) (OVX + Si group). A group of 15 OVX rats and the group of 15 sham-OVX rats did not receive the above supplements. The water consumption, body weight, and food intake were measured twice a week in sham-OVX rats with the same amount of food given to ovariectomized animals (pair feeding) to avoid hyperphagia and overweight associated with ovariectomy.

Morphometric and Densitometric Studies

At the conclusion of the 30-day experiment, the rats were sacrificed by exsanguination from the abdominal aorta after being anesthetized with 4 mg/100 g body weight of sodium pentothal. Success of ovariectomy was confirmed at necropsy by failure to detect ovarian tissue and by observing marked atrophy of the uterine horns. The rat femurs and 5th lumbar vertebrae were dissected and soft tissue was removed. Femoral and vertebral length were measured with a caliper and bones were weighed on a precision balance. The bone mineral content (BMC) and BMD of the whole femur (F-BMC mg and F-BMD mg/cm^2) and 5th lumbar vertebra (V-BMC mg and V-BMD mg/cm^2) were measured separately. Because of the influence of weight on bone mass [11], femur BMC was corrected for the animals final body weight (femur BMC/FW mg/g). As in our earlier studies [11–14], we have relied on DXA (Norland XR-26, Norland Co., Fort Atkinson, Wisconsin, USA). Our coefficients of variation (CV) for the current measurements, determined by six separate measurements done on three rat femurs and 5th vertebras at intervals of 3–4 days were 0.8% and 0.7%, respectively. The instrument was calibrated daily.

Statistical Studies

Descriptive statistics are presented as mean ± SD. The normal distribution of data was confirmed by calculating skew and kurtosis before applying standard tests. The studied parameters (continuous variables) in each group (nominal variables) were compared using analysis of variance (ANOVA) and covariance to determine the effects of nominal variables; data were analyzed by ANOVA with a post hoc test of differences among groups using the Bonferroni/Dunn test. A minimum *P*-value of 0.005 was the necessary condition for statistical significance. Data were processed on a Macintosh computer using the StatView 4.02 statistical package (Abacus Concepts, Berkeley, CA, USA).

Results

Group characteristics (number, baseline, and final body weight, anthropometric data, etc.) are summarized in Table 1. As it can be observed, the initial weight did not differ among the three rat groups, but it had increased significantly in the OVX and Si+OVX groups at the end of the study when compared with the sham-OVX ($P < 0.0001$) group. The femur length (mm) and its weight had decreased in the OVX group contrary to the other two groups ($P < 0.0001$). A similar change was observed for F-BMC mg, V-BMC mg, F-BMD mg/cm^2, V-BMD mg/cm^2 as well as in vertebral weight and F-BMC/FW mg/g ($P < 0.0001$ in all). Subsequently, the only parameters where no differences were observed were the length or height of the 5th lumbar vertebra as studied.

Discussion

Our results showed that Si inhibits the loss of bone mass in rats subjected to OVX and that it promotes the longitudinal growth of long bones, in this case, the femur. On the other hand, Si incremented corporal mass by 11.5%. It was shown that OVX induces a deficit of longitudinal growth in bones

[15] and that Si is very important for it sanctions the longitudinal development, as claimed by Schwart and Milne [4] and later confirmed by Carlisle [5]. Sontag [16] has reported that at 150 days of life, femoral length in female Wistar rats reached a mean of ± 34 mm. In our study the longitudinal development of the femur in the Si-OVX group was notably enhanced. This phenomenon may be related to the mentioned effect of Si, that it promotes bone growth [4], which has also been certified by most recent studies [17]. The mentioned reports showed that an aluminum-silicon mixture increases the proliferation and differentiation of osteoblasts. It is an action that can be dependent on the mentioned mixture, known otherwise as Zeolite A, which in turn exerts effect on TGFb, a citoquine that stimulates bone formation *in vivo* [18]. This action is not to be linked to the aluminum by the toxic effect of aluminum on the bone [19].

The increase in the body weight of rats treated with Si supplement was also patent, which fully coincided with the reports of other authors [4, 5], even though their claims inferred greater proportion, as the one observed by us. Nonetheless, it once again proved the positive effect of Si on the rats' somatic development. The rats' weight in this study was significantly correlated with bone mass [11]. When the final bone mass, such as femur BMC (F-BMC mg) was divided by the final rats' weight (F-BMC/FW mg/g), the results were the same at significant levels, closely matching those observed in the study on femurs. This fact alone vouches for the importance that the Si exerts on the prevention of bone loss induced by OVX.

It is widely held that experimental oophorectomy of laboratory animals supposedly mimics the sequence of events that take place in human subjects following menopause. In the presently oophorectomized rats, bone mass loss was rapid. Ovariectomy results in severe cancellous osteopenia of murine long bones and vertebras [15]. A similar phenomenon was observed in our study, where the loss due to OVX was evident in the axial skeleton (vertebra), reaching 30% and 25% in the periphery (femur), respectively. There is ample evidence that after immediate menopause and/or OVX, the loss is greater at the axial skeleton than in the periphery [20], which was possible to confirm in the present study. Nonetheless, the observed significant effect on Si on the bone mass has to do with the prevention of post-OVX losses, which is known to be secondary to an excessive bone resorption.

Based on results concerning longitudinal development in the peripheral skeleton, we are in a position to conclude that Si has a very important effect on the stimulation of bone formation. It is also obvious that these results need to be related to the recent studies conducted by Hott et al. [21] and Schutze et al. [22] which demonstrate that Si decreases the osteoclast number by 20% and that Si exerts a potent inhibitory effect on bone resorption while increasing the rate of bone formation [21]. These findings coincide with Eisinger and Cairet's studies [23] which show that Si is a more potent bone formation stimulus than other drugs, such as ethidronate, used in the treatment of osteoporosis. The double action to which we refer—on one hand, a potent inhibition of the bone resorption and on the other a significant stimulation of bone formation—suggests that Si assumes two crucial functions in the treatment of osteoporosis. The forthcoming conclusion was amply justified by the good results obtained in the present study.

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