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Pathology

Comparison between two experimental protocols to promote osteoporosis in the maxilla and proximal tibia of female rats

Comparação entre dois protocolos experimentais para promover osteoporose no osso maxilar e na tíbia proximal de ratas

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ABSTRACT: The effects of two experimental protocols (ovariectomy associated or not with a low calcium diet) used to promote osteoporosis in the rat maxilla and proximal tibia were compared 5 and 11 weeks after surgery. Female Wistar rats were ovariectomized or sham-operated. Half of the ovariectomized rats were fed a low Ca^{++} diet (ovx*) and the remaining ovariectomized (ovx) and sham animals received a standard chow. At sacrifice, the proximal metaphysis was excised from the tibia and the molars were extracted from the hemi-maxilla. Dry (60°C/overnight) and ash (700°C/14 h) weights were measured and the ashes were used for Ca^{++} measurement by means of a colorimetric method. After 5 weeks, ovx caused no alteration while ovx* decreased proximal metaphysis (17%) and maxilla (35%) bone mass. After 11 weeks, ovx caused a 14% bone mass reduction in the proximal metaphysis but not in the maxilla, while ovx* caused a comparable bone mass reduction (30%) in both bone segments. Calcium concentration was not altered in any experimental condition. The results show that estrogen deficiency is insufficient to cause maxillary osteoporosis in rats over an 11-week period and a long-term ovariectomy is needed to exert deleterious effect on proximal metaphysis bone mass. When a low Ca^{++} diet is associated with estrogen deficiency, however, a relatively precocious harmful effect is observed, twice as pronounced in the maxilla than in the proximal metaphysis. On a long-term basis, ovariectomy associated with a low Ca^{++} diet seems to be equally injurious to both proximal metaphysis and maxilla.

DESCRIPTORS: Osteoporosis; Tibia; Maxilla; Ovariectomy.

RESUMO: Comparou-se o efeito de dois protocolos experimentais (ovariectomia associada ou não à dieta pobre em Ca^{++}) utilizados para promover osteoporose em maxila e metáfise proximal de ratas, nos períodos de 5 e 11 semanas pós-cirurgia. Ratas Wistar foram ovariectomizadas ou submetidas à cirurgia simulada. Metade das ratas ovariectomizadas recebeu dieta pobre em Ca^{++} (ovx*) e as demais (ovx), assim como as que sofreram falsa cirurgia, receberam dieta comercial. Foram coletados o osso maxilar (após extração dos molares) e a metáfise proximal da tíbia para medidas do peso seco (60ºC/12 h) e da cinza óssea (700ºC/14 h), utilizada para dosagem de Ca^{++} (método colorimétrico). Cinco semanas após a cirurgia, não se observaram alterações nos parâmetros investigados no grupo ovx, enquanto no grupo ovx* houve redução da massa óssea da metáfise proximal (17%) e da maxila (35%). Após 11 semanas, o grupo ovx apresentou 14% de redução da massa óssea da metáfise proximal, mas não da maxila, enquanto no grupo ovx* observou-se diminuição de 30% em ambos os segmentos ósseos. A concentração de Ca^{++} na cinza não se alterou em nenhuma condição experimental. Os resultados mostram que apenas a deficiência de estrógeno não é suficiente para provocar osteoporose maxilar em ratas num período de até 11 semanas, mas que nesse período já se observa seu efeito deletério na massa da metáfise proximal. Quando se associa a ovariectomia à dieta pobre em Ca^{++}, observa-se diminuição da massa óssea após 5 semanas, 2 vezes maior na maxila do que na metáfise proximal da tíbia.

DESCRITORES: Osteoporose; Tíbia; Maxila; Ovariectomia.

INTRODUCTION

Ovariectomized rats have been widely used as an animal model to simulate human post-menopausal accelerated bone loss. Besides estrogen deficiency, a decrease in intestinal calcium (Ca^{++}) absorption also occurs with aging and may contribute to the accompanying bone loss, which results in osteoporosis when bone mass falls to a level at which it is more susceptible to fracture[12].

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Thus, a low Ca^{++} diet accompanied or not by ovariectomy has been sometimes utilized as an experimental protocol to simulate human osteoporosis[7,12,14-16,19].

The literature shows that the effects of estrogen deficiency on bone characteristics such as size, mass and density are site-dependent, with the cancellous appendicular (proximal femur and tibia) and axial (vertebra) bones being by far those most investigated for osteopenia due to a higher incidence of spontaneous fractures observed at these skeletal sites. There is comparatively little information about the incidence of increased alveolar bone loss in estrogen-deficient women[8,11,13,20,21] and the animal studies conducted to investigate whether alveolar bone may also be influenced by factors causing systemic osteoporosis have produced conflicting results[2,5,7,9,10,14,18,19,22,23,25].

Considering that contradictory results may arise from different experimental designs, the purpose of the present study was to compare the effects of two protocols (ovariectomy associated or not with a low calcium diet) used to promote osteoporosis in the maxillar alveolar bone and proximal tibia after shorter (5 weeks) and longer (11 weeks) periods of treatment.

MATERIAL AND METHODS

Female Wistar rats (209.7 ± 4.2 g initial body weight) were bilaterally ovariectomized (ovx, n = 40) or sham-operated (sham, n = 20) under 2,2,2, tribromoethanol anesthesia (Aldrich, Milwaukee, USA; intraperitoneal injection of 25 mg/100 g body weight). A single intramuscular injection of a polyvalent veterinary antibiotic (Pentabiótico Veterinário, Wyeth, São Bernardo do Campo, SP, Brazil; 0.2 ml/rat) was administered immediately after surgery. Half of the ovx rats received a low calcium (0.1% Ca^{++}) and phosphorus (0.5% P) diet (Rhoster Ind. Com., Vargem Grande Paulista, SP, Brazil) from the day of surgery to sacrifice, while the remaining ovx animals as well as those from the sham group were fed a standard laboratory chow (Nuvilab, Curitiba, PR, Brasil) containing 1.4% Ca^{++} and 0.8% P. The animals were housed in a climate-controlled environment (temperature 23 ± 2ºC, light cycle with 12 h light beginning at 6:00 a.m.) and received a solid diet and tap water *ad libitum*.

The rats were killed with an intraperitoneal overdose of sodium pentobarbital 5 or 11 weeks following surgery (n = 10 per group), their left tibia and hemi-maxilla were removed, freed of soft tissues and stored at -20ºC until the measurements were performed. The molars were extracted from the left hemi-maxilla and the corresponding region of the alveolar process (from the mesial face of the first molar to the distal face of the third molar) was also resected for analysis. The bone segments were maintained overnight at 60ºC for the determination of dry weight (organic + mineral contents) and then ashed at 700ºC for 14 h. After weighing, the ashes (mineral content) were used for measurement of Ca^{++} by a colorimetric method (Spectrophotometer B380, Micronal, São Paulo, Brasil) using specific commercial kits (Labtest Sistemas Diagnósticos Ltda., Belo Horizonte, MG, Brazil).

Statistical analysis

Differences between groups were analyzed by the non-parametric Kruskal-Wallis test.

RESULTS

The results (Table 1, summarized in Table 2) showed that estrogen deficiency resulting from 5 weeks of bilateral ovariectomy was ineffective in promoting any significant alteration in either the proximal metaphysis or maxillary bone mass (estimated by dry and ash weights in addition to total Ca^{++} amount). During the same period, a low Ca^{++} diet associated with ovariectomy resulted in a significantly decreased bone mass in the proximal metaphysis (17%) and maxilla (30%-40%).

Following a longer post-ovariectomy period (11 weeks), estrogen deficiency resulted in a slight bone mass reduction in the proximal metaphysis (14% decrease in the dry weight, while ash weight and Ca^{++} content tended to show a non significant decrease). These changes were not observed in the maxilla.

A low Ca^{++} diet provided to ovariectomized rats for 11 weeks caused an apparently comparable bone mass reduction in both the proximal metaphysis (a decrease of 23% and 27% in dry and ash weights, and of 32% in Ca^{++} amount) and maxilla (a decrease of 31% and 28% in dry and ash weights, and of 32% in Ca^{++} amount). Calcium concentration in the proximal metaphysis and maxilla of ovariectomized rats (ovx and ovx* groups) did not differ significantly from that of sham animals.

DISCUSSION

Although osteoporosis is a significant public health problem and despite the presumed risk of oral bone loss accompanying postmenopausal os-

TABLE 1 - Proximal metaphysis and maxilla from sham-operated rats (sham), ovariectomized rats receiving a standard laboratory chow (ovx) and ovariectomized rats receiving a low Ca^{++} diet (ovx*). The results are expressed as mean ±SEM (standard error of the mean). For each post-surgery period and each bone parameter, different letters denote statistically significant differences between the experimental groups (Kruskal-Wallis test, $A \neq B \neq C$, $\alpha = 0.05$).

Parameters	5 weeks post-surgery			11 weeks post-surgery		
	Sham	Ovx	Ovx*	Sham	Ovx	Ovx*
	Proximal metaphysis					
Dry weight (mg)	88.0 ± 3.9$^{(A)}$	91.8 ± 2.8$^{(A)}$	73.8 ± 2.2$^{(B)}$	129.6 ± 5.1$^{(A)}$	111.5 ± 2.6$^{(B)}$	100.1 ± 2.0$^{(C)}$
Ash weight (mg)	47.2 ± 2.0$^{(A)}$	47.6 ± 1.2$^{(A)}$	39.3 ± 1.5$^{(B)}$	74.4 ± 6.8$^{(A)}$	58.1 ± 1.6$^{(AB)}$	54.5 ± 2.5$^{(B)}$
Total Ca^{++} content (mg)	38.4 ± 3.6$^{(AB)}$	39.3 ± 2.0$^{(A)}$	31.7 ± 2.3$^{(B)}$	64.3 ± 7.3$^{(A)}$	47.4 ± 3.1$^{(AB)}$	43.7 ± 4.0$^{(B)}$
Ca^{++} concentration (mg/mg ash)	0.79 ± 0.04$^{(A)}$	0.82 ± 0.05$^{(A)}$	0.81 ± 0.05$^{(A)}$	0.85 ± 0.05$^{(A)}$	0.81 ± 0.04$^{(A)}$	0.79 ± 0.04$^{(A)}$
	Maxilla					
Dry weight (mg)	64.2 ± 1.6$^{(A)}$	65.6 ± 3.2$^{(A)}$	44.3 ± 1.8$^{(B)}$	74.5 ± 4.9$^{(A)}$	66.8 ± 4.0$^{(A)}$	51.6 ± 2.7$^{(B)}$
Ash weight (mg)	43.4 ± 1.5$^{(A)}$	37.9 ± 4.0$^{(AB)}$	30.7 ± 2.6$^{(B)}$	51.7 ± 3.2$^{(A)}$	45.4 ± 3.0$^{(A)}$	37.2 ± 1.7$^{(B)}$
Total Ca^{++} content (mg)	34.8 ± 0.7$^{(A)}$	29.7 ± 2.9$^{(A)}$	20.7 ± 2.9$^{(B)}$	43.4 ± 3.3$^{(A)}$	39.9 ± 3.1$^{(A)}$	29.4 ± 2.2$^{(B)}$
Ca^{++} concentration (mg/mg ash)	0.80 ± 0.03$^{(A)}$	0.80 ± 0.05$^{(A)}$	0.66 ± 0.06$^{(A)}$	0.83 ± 0.01$^{(AB)}$	0.87 ± 0.02$^{(A)}$	0.79 ± 0.03$^{(B)}$

TABLE 2 - Bone mass decrease (estimated by mean percent reduction of dry and ash weights and total Ca^{++} amount) in the proximal metaphysis and maxilla of ovariectomized rats receiving a standard laboratory chow (ovx) and ovariectomized rats receiving a low Ca^{++} diet (ovx*), as compared to sham-operated animals.

Bone	5 weeks post-surgery		11 weeks post-surgery	
	Ovx	Ovx*	Ovx	Ovx*
Proximal metaphysis	≈	↓ 17%	↓ 14%	↓ 28%
Maxilla	≈	↓ 35%	≈	↓ 30%

≈ (similar) or ↓ (decreased) in comparison with the sham group.

teoporosis, this correlation still lacks confirmation. In edentulous women with osteoporotic fractures, symptomatic osteoporosis seems to be a severe risk factor for residual ridge reduction of the maxilla but not of the mandible[24]. An association between skeletal osteoporosis and decreased mandibular bone density has been suggested by some investigators but not recognized by others[18]. A critical review shows that technical difficulties besides inadequate experimental designs have made it difficult to draw conclusions about this topic[4]. Thus, an adequate animal model of oral bone osteoporosis, to be used to investigate the implications of clinical procedures such as tooth extraction, implants, bone grafting, ridge augmentation, in addition to pathological processes such as periodontal disease and the efficacy of therapeutic agents against oral bone loss, would be advantageous.

Ovariectomized animals have been helpful in providing an insight regarding human postmenopausal osteoporosis because both share many characteristics including an increased rate of bone turnover with resorption exceeding formation. However, the deleterious effect of ovariectomy on oral bones remains unconvincing. Elovic *et al.*[2] examined the long-term effect (up to 200 days) of ovariectomy on the rat mandible. Since the experiment included both adult and old rats, the effect of aging in addition to ovariectomy could also be identified. The authors concluded that estrogen depletion contributes to oral bone loss, an effect that may be accentuated by aging. The effect of aging and ovariectomy was also investigated on the rat mandibular condyle and no significant alteration in bone mineral density was found by dual-energy X-ray absorptiometry up to 60 days post-ovariectomy, probably because the thickness of cortical bone obscured any possible change in trabecular bone. However, estrogen deficiency seemed to cause a significantly large marrow area, allowing the authors to speculate that osteoporotic changes may occur in the mandibular condyle[22].

A histometric analysis performed 2 months post-ovariectomy showed no significant change in

the amount of compact or trabecular bone in the mandible and only a 10-25% increase in bone porosity in the maxilla[25]. The authors concluded that rats, due to their peculiar masticatory habits placing large loads on oral bones, are not a suitable experimental model for studying oral bone loss related to skeletal osteoporosis and that, to worsen oral osteopenia, it would be mandatory to combine ovariectomy with a mechanical unloading, i.e. after molar extraction. In fact, ovariectomy-induced estrogen depletion has been shown to affect bone healing/remodeling after molar extraction by increasing bone resorption and reducing bone formation, an effect observed earlier in the maxilla[9,10,17,18] than in the mandible[3,4]. Compared to the distal femur, the changes in the edentulous mandible of ovariectomized rats take longer, possibly due to a larger proportion of trabecular bone composing the femur, while the edentulous mandible contains primarily cortical bone[4].

In this respect, literature data have shown that the maxillary cortical bone shell, like the mandibular one, is not markedly influenced by short- or long-term estrogen depletion[18]. The reason why trabecular bone is lost faster than cortical bone is that trabecular bone turnover is greater than cortical turnover due to the much greater number of bone cells and larger surface area in the former[6].

The present results support literature data showing that the metaphysis of a long bone is more affected by estrogen deficiency than the oral bone and that ovariectomy alone is not effective in affecting maxillary bone mass, at least over an 11-week period.

Additionally, a low Ca^{++} diet has been used as a protocol for oral osteoporosis in female rats. A Ca^{++} diet reduced to 6% of the control one, administered for 16 weeks, caused practically the same rate of reduction in the cancellous bone of the proximal tibia, first tail vertebra and mandible[15]. The authors emphasized that a common denominator of all bone segments investigated was that they contained cancellous bone readily available for the maintenance of calcium homeostasis.

A combined ovariectomy and low Ca^{++} diet is an experimental design seldom used to investigate osteoporosis in oral bones. Moriya *et al.*[14] compared by radiographic and visual inspection the bone mineral density and bone loss in rat femur, tibia, maxilla and mandible following ovariectomy and/or a low (0.005%) Ca^{++} diet administered for 4 weeks. Although bone mineral density was decreased in all bones by a low Ca^{++} diet associated or not with ovariectomy (but not by ovariectomy alone), no significant alteration was detected regarding alveolar bone loss (evaluated by the distance from the cemento-enamel junction to the bone crest at the center of the molars mesial root). Contrarily, a significant increase in both bone formation and resorption, resulting in a decreased bone volume, were detected by histomorphological analysis applied to the cortical maxillary bone and to the cancellous bone of mandible and proximal tibia, from 12 to 32 weeks post-ovariectomy associated to a low (0.02%) Ca^{++} diet[19].

The present results show that feeding ovariectomized rats a low (0.1%) Ca^{++} diet caused a bone mass reduction which was slight in the proximal metaphysis but more pronounced in the maxilla, as early as during the 5th week post-surgery, although on a long-term basis the treatment seemed equally injurious to both bones. It has been demonstrated that dietary Ca^{++} deficiency seems to induce bone loss in both cortical and cancellous bone whereas bone loss due to estrogen deficiency is mainly confined to cancellous bone[16]. Moreover, rats fed a diet containing more than 1% Ca^{++} (as is the case for most standard chows) have reduced bone sensitivity to ovariectomy, whereas in ovariectomized animals fed a low Ca^{++} diet the decrease in Ca^{++} absorption due to ovariectomy becomes significant and bone loss is enhanced[12].

In the present study, Ca^{++} concentration in the proximal metaphysis and maxilla of ovariectomized rats receiving or not a low Ca^{++} diet did not differ significantly from that of sham-operated animals. It has been shown that ovariectomy in rats, like the postmenopausal period in women, results in loss of bone matrix with no alteration in bone matrix mineralization[6]. Osteoporosis has been defined as a generalized, progressive diminution in bone mass, causing weakness of skeletal strength, even though the ratio of mineral to organic elements is unchanged in the remaining normal bone[1]. A low bone mass accompanied by trabecular disruption and cortical porosity is seen in osteoporosis, in contrast to an equally low bone mass with disturbances in mineralization observed in osteomalacia[4].

CONCLUSION

In conclusion, the animal model of a low Ca^{++} diet administered to rats with ovariectomy-induced estrogen deficiency described here proved to be an effective protocol for maxillary osteoporosis, even during a short-term experimental period, promising to be useful in future investigations regarding the implications of clinical procedures, pathological processes and the efficacy of therapeutic agents against oral bone loss.

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