Periodontal & systemic bone changes in rats with experimental lathyrysm

Gonca Keles, Tarik Basoglu*, Oktay Yapici*, Burcu Ozkan Cetinkaya, Gokhan Acikgoz & Erhan Fıratlı**

Ondokuzmayıs University, Faculty of Dentistry, Department of Periodontology, Faculty of Medicine, Department of Nuclear Medicine, Samsun & University of Istanbul, Faculty of Dentistry, Department of Periodontology, Istanbul, Turkey

Received May 19, 2005

Background & objectives: It is not clear how lathyrysm affects the systemic bone metabolism. We therefore undertook a study to observe periodontal and systemic bone changes by performing radiological, metabolic, and bone densitometric evaluations in rats with experimental lathyrysm.

Methods: A total of 30 rats were used. Experimental lathyrysm was induced by once daily subcutaneous administration of beta-aminopropionitrile (β-APN), at a dose of 5 mg β-APN/0.4 ml per 100 g of body weight for 40 days. After 40 days, vertebral bone mineral density was analyzed by means of dual energy X-ray absorbtometry in both groups. Blood was drawn by cardiac puncture and the animals were decapitated. Serum calcium levels were measured. Right mandibles were removed and radiographs were obtained. Alveolar bone level was determined in the radiographs.

Results: In all lathyritic rats, alveolar bone level was pathologically decreased with visible resorption. Vertebral bone mineral density values of lathyritic rats did not differ significantly from those of the control group. Compared to controls, there was a statistically significant decrease in serum calcium levels in the lathyritic group (P<0.001).

Interpretation & conclusion: Significant alveolar bone resorption without alterations in vertebral bone mineral density indicated that lathyrogen administration for 40 days presumably has not caused systemic demineralization. This model could be used for studying the role of local and systemic agents on periodontal alveolar bone resorption.

Key words Bone mineral density - bone resorption - calcium - lathyrysm - periodontitis

Osteolathyrysm, a connective tissue disease, is characterized by decreased intramolecular and intermolecular cross-linking of collagen molecules1-3. Lathyrogenis inhibit lysyl oxidase, which catalyzes the conversion of lysine and hydroxyllysine into an aldehyde group and the formation of collagen cross-linking4,5. The lathyritic agent beta-aminopropionitrile (β-APN) first isolated
from the sweet pea, *Lathyrus odoratus*, by Selye in 1957, is considered an appropriate agent for studying connective tissue metabolism. Periodontal disease is an inflammatory disease of periodontal tissues and results in loss of tooth supporting tissues, including alveolar bone. Alveolar bone resorption is an important feature of periodontal disease. However, it is suggested that a decrease in the bone mineral content of the skeleton may aggravate periodontal disease.

The organic matrix of cortical bone principally consists of fibrous protein collagen, as well as acidic glycoproteins, serum proteins, and proteoglycans. Collagen forms 90 per cent of the organic matrix of bone and has a major function in calcification. Calcium phosphate crystals usually form on collagen fibrils.

Bone demineralization is characterized by the activity of osteoclasts. During the resorptive phase of bone metabolism, the inorganic constituents, collagen fibers and organic substance are lost. The effect of lathyrogens on bone metabolism or calcium homeostasis is not well understood though their effects on the organic portion of the bone are well known. Only one study reporting demineralization in lumbar vertebrae of lathyritic rats could be found in the earlier literature.

β-APN produces lathyritic changes in bone and periodontal ligament collagen in an average period of three weeks at any dosage. In our previous experimental study, significant alveolar bone resorption was detected radiographically and histopathologically at 40 days. No information is available on the determination of systemic bone changes by dual energy X-ray absorptiometry (DEXA), the most reliable method of measuring bone mineral density, in experimental lathyrism.

The present study was undertaken to observe periodontal and systemic bone changes by performing radiological, metabolic, and bone densitometric evaluations in rats with experimental lathyrism following 40 days of β-APN administration. Besides millimetric measurements of alveolar bone level on X-ray films and determination of serum calcium levels, it was planned to measure the systemic bone response to lathyrogen administration on vertebrae using DEXA.

### Material & Methods

This study was done at the Ondokuzmayis University Medical and Surgical Research Center, Samsun, Turkey, in 2001. Male, adult Wistar rats (n=30) with an average weight of 200-300 g, were used in the study. They were housed separately in plastic cages and kept in a temperature-controlled room with a standard 12:12 h light-dark illumination cycle. In the lathyritic group (n=15), experimental lathyrysm was induced by once daily subcutaneous administration of β-APN (Sigma-Aldrich Chemie., Taufkirchen, Germany), at a dose of 5 mg b-APN/0.4 ml distilled water per 100 g of body weight for 40 days. Control rats (n=15) received daily subcutaneous injections of 0.4 ml/100 g body weight of saline. All rats were fed a powdered diet and water *ad libitum*.

At the end of the 40 days, analysis of the bone mineral density (BMD) by means of DEXA was performed in both groups under systemic anesthesia obtained by the intraperitoneal administration of ketamine-HCl (Warner Lambert, Pfizer Inc., Istanbul, Turkey), at a dose of 60 mg/kg. A dual-energy X-ray bone densitometer with Samarium filter producing two energy peaks at 46.8 and 80 KeV was used in the study. BMD was calculated using a rectangular region of interest drawn over the mid-portion of the lumbar vertebral column.

Following blood withdrawal by cardiac puncture, all animals were decapitated. Their right mandibles were carefully removed, and radiographs were obtained by long cone technique at 70 KvP, 8 mA (Trophy Dental Radiography, Istanbul, Turkey). Alveolar bone loss, as the distance between the radiographic cemento-enamel junction to the alveolar crest, was determined in radiographs using millimeter-scaled paper. Radiographic measurements were performed on both the mesial and distal aspects of the mandibular molar teeth.
Blood samples were centrifuged at 500 x g for 10 min to obtain sera. Serum calcium levels were measured by Roche-Hitachi MODULAR system (Roche Diagnostic, Mannheim, Germany). Determination of serum calcium was based on the reaction of calcium with o-cresol-phtalein complexone in an alkaline solution\textsuperscript{21}. Magnesium was masked with 8-hydroxyquinoline. The colour intensity of the purple complex formed is directly proportional to the calcium concentration and is measured photometrically. Two point calibrations were performed.

The study protocols were approved by the Animal Experiment Ethics Committee of Ondokuzmayis University, Turkey.

The statistical analysis was performed using a commercially available software programme (SPSS 12.0, SPSS Inc., Chicago, Illinois, USA). When measuring the alveolar bone level, only recordings representing the deepest site in each rat were used in the statistical analysis. Shapiro Wilk test was used the data were normally distributed. For the statistical comparison of the two relevant groups student t-test was used. \( P<0.05 \) was considered as significant.

**Results & Discussion**

In the lathyritic rats, alveolar bone level was pathologically decreased with visible resorption. The mean value of alveolar bone loss in the study group was 2.133 ± 0.15 mm. In the visual evaluation of the X-ray films of the control group, no measurable alveolar bone resorption could be detected (Figs 1 and 2).

The mean values of vertebral BMD were 0.1432 ± 0.001 g/cm\textsuperscript{2} in the study group and 0.1414 ± 0.001 g/cm\textsuperscript{2} in the control group. There was no statistically significant difference in vertebral BMD between the two groups. Compared to controls, there was a statistically significant decrease in serum calcium levels in lathyritic rats (\( P<0.001 \)). The serum calcium levels were 10.3453 ± 0.09 mg/dl in the lathyritic group and 11.8393 ± 0.11 mg/dl in the control group.

![Fig. 1. Radiographic alveolar bone loss which is between cemento-enamel junction and the most apical alveolar bone in the lathyritic group (short arrows \( \rightarrow \), \( \leftarrow \): cemento-enamel junction; long arrows \( \rightarrow \), \( \leftarrow \): Alveolar crest).](image1)

![Fig. 2. Radiographically healthy periodontium in the control group.](image2)
These findings demonstrated a significant alveolar bone loss in the lathyritic rats. No systemic effects on bone mineral status could be detected by vertebral BMD measurements. After administration of the lathyritic agent, β-APN during a period of 40 days, the mandible was significantly affected with measurable alveolar bone resorption on X-ray films of all lathyritic animals. This result was in accordance with our previous study that showed alveolar bone loss after 40 days of lathyrogen administration.18 At six weeks, significant osteoclastic resorption of alveolar crest has been demonstrated histopathologically in experimental lathyrism. Alveolar bone is subjected to occlusal forces, which gives it a dynamic nature and mineral exchange. During this process, the alveolar bone may be affected by various products, such as lathyrogens.

DEXA studies performed on alveolar bone sections of dogs have shown that, this method is able to provide precise and accurate measurement of BMD changes as small as 1 per cent in histological bone areas of 3.1 mm². Since the mandibular bone components of rats are much smaller in size, performing BMD measurements without methodological validation was considered inaccurate. However, the vertebral corpus size of rats is in an acceptable range as shown in former validation trials. Bone mineral density of the vertebrae, as measured by DEXA, is a sensitive parameter because of the higher metabolic activity and greater response to osteoporosis in these bones.21-22 The vertebral corpus of rats is easy to delineate by DEXA, which also minimizes operator error.

In the present study, vertebral demineralization was not detected following 40 days of lathyritic agent administration. In an experimental study, computer-aided photometrical measurements of mineral densities in the lumbar vertebrae of lathyritic rats on microradiographs were performed and findings of bone demineralization were reported. The author concluded that mineralization defect was related to disturbed collagen synthesis in lathyrysm.23

In a comparative human trial involving the alveolar bone crest and lumbar spine, interestingly, a similar sensitivity of both osseous sites to hormone therapy was observed. In postmenopausal women receiving estrogen replacement therapy, the authors measured alveolar bone crest height on bite-wing radiographs, determined changes in alveolar bone mass by means of digital subtraction radiography, and measured BMD in lumbar spine and proximal femur by DEXA. They observed a significant increment of alveolar bone mass and femoral BMD, but not BMD of the lumbar spine. They concluded that therapy tended to improve alveolar crest height without any changes in the BMD of the lumbar spine.24 Another study reported that bone loss affected the jaw bones (particularly the alveolar bone), cranial bones, ribs, vertebrae, and long bones in descending order.25

Although the main effect of lathyrogens was observed on the organic portion of the bone, in the present study, both alveolar bone resorption and a significant decrease in serum calcium levels were observed in lathyritic rats. The reduced calcium levels observed in the lathyritic group could be related to the poor calcium absorption caused by β-APN. Inhibition of calcium absorption in lathyrogen-exposed (β-APN and semicarbazide) chicks has been demonstrated in an experimental study.26

It is known that low serum calcium promotes parathyroid hormone secretion, resulting in bone resorption.27,28 We may speculate that in our study, alveolar bone resorption might have begun due to poor calcium absorption. In an earlier study lower total serum calcium levels were shown to be associated with more alveolar bone loss in periodontal disease.29

To conclude, in lathyritic rats, significant alveolar bone resorption without alterations in vertebral BMD was observed. These results indicated that lathyrogen administration for 40 days presumably did not cause systemic demineralization in rats. Local bone resorption at the alveolar level following 40 day β-APN administration, could be demonstrated by means of plain X-ray radiography. This experimental model may be used for studying the role of local and systemic agents on periodontal alveolar bone resorption.
References


Reprint requests: Dr Gonca Keles, Ondokuzmayis University, Faculty of Dentistry
Department of Periodontology, Samsun, Turkey
e-Mail: goncakeles@hotmail.com; goncak@omu.edu.tr