AN EXPERIMENTAL MODEL OF DISUSE IN THE ALVEOLAR RAT BONE. A HISTOMORPHOMETRICAL STUDY

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ABSTRACT

Lack of mechanical stimulation is known to cause disuse osteopenia in bones. However, experimental models for disuse osteopenia on bones other than jawbones are not applicable to jawbones. The little available information in this field has been applied to the study of overeruption of teeth lacking antagonists. However, the absence of an antagonist in the opposite jaw means that there is no stimulation by occlusion. Our hypothesis is that the lack of stimulation due to the absence of teeth causes disuse osteopenia in the interradicular bone of the antagonist teeth. Our aim was to develop a model of disuse osteopenia due to the absence of occlusal forces. We used male Wistar rats with 215-230 g body weight, divided into 2 groups: one absolute control group (C) and one experimental group in which the three right lower molars were extracted (E). The left side of the jaw in the experimental group was used as a paired control (PC). The animals were euthanized 7 days after extraction. The jaws were placed in occlusion, fastened and fixed in 10% formalin. The heads were cut in half and radiographs made of both jaws. The upper jaws were processed histologically. After decalcification, bucco-palatine oriented sections were cut at the level of the mesial root and distal roots of the first upper molars. On the radiographs, the distance from the tip of the cusp on the first upper molar to the antagonist edentulous ridge (DA) was measured. On the microphotographs, the following parameters were measured: passive eruption degree (PED), height of periodontal ligament at the level of the furcation (HPL) and interradicular bone volume (BV_I). The data were compared statistically using ANOVA and Bonferroni's post-hoc test, considering $p< 0.05$ as statistically significant. DA in experimental animals was 0.34±0.048 mm. PED in experimental animals was significantly greater than in the control groups, both for the buccal plate and for the palatal plate. HPL showed no significant difference between groups. BV_I was significantly lower in the experimental group than in the control group. The results showed that the model used produces a condition of disuse osteopenia, shown by the statistically significant reduction in interradicular bone volume. The use of this model at different experimental times will enable the evaluation of cell responses in periodontal tissues, particularly bone tissue, e.g. to compare them to known responses such as the application of orthodontic forces.

Key words*: disuse, experimental model, alveolar bone.*

MODELO EXPERIMENTAL PARA EL ESTUDIO DEL EFECTO DEL DESUSO EN EL HUESO MAXILAR SUPERIOR DE RATA

RESUMEN

Se sabe que la falta de estímulo mecánico produce un cuadro de osteopenia por desuso en huesos de la economía. Los modelos experimentales de osteopenia por desuso utilizados en otros huesos no son aplicables a los huesos maxilares. La escasa información que existe en este campo se aplicó al estudio de la sobreerupción de dientes sin antagonista. Sin embargo, la ausencia de antagonista en el maxilar opuesto hace que falte el estímulo de la oclusión. Por tal razón, nuestra hipótesis es que la falta de estímulo por ausencia de piezas dentarias provoca un cuadro de osteopenia por desuso en el hueso interradicular de los dientes antagonistas. Nuestro objetivo fue poner a punto un modelo de osteopenia por desuso debido a la ausencia de fuerzas oclusales. Se emplearon ratas Wistar machos de entre 215-230 g de peso corporal divididas en 2 grupos, un grupo control absoluto (C) y un grupo experimental al que se le extrajeron los tres molares inferiores derechos (E). El lado izquierdo del maxilar del grupo experimental, fue utilizado como control apareado (CA). A los 7 días de efectuadas las extracciones se realizó la eutanasia de los animales. Los maxilares se colocaron en oclusión, se precintaron y así ubicados se fijaron en formol 10%. Se dividieron las hemicabezas y tomaron radiografías de ambos maxilares siendo procesados histológicamente los maxilares superiores. Luego de la descalcificación se obtuvieron cortes orientados en sentido buco-palatino a nivel de la raíz mesial y de las raíces distales de los 1°molares superiores. Sobre las radiografías se midió la distancia desde el vértice cuspídeo del 1° molar superior al reborde antagonista desdentado (DA), y sobre microfotografías se midieron los siguientes parámetros: grado de erupción pasiva (GE), altura del ligamento periodontal a nivel de la furcación (ALP) y volumen óseo interradicular (VO_I). Los datos se compararon estadísticamente mediante el test ANOVA y prueba post hoc de Bonferroni, considerando una $p< 0,05$ como estadísticamente significativa. La DA en los animales experimentales fue de 0,34±0,048 mm. La GE en los animales experimentales fue significativamente mayor que en los grupos control, tanto a nivel de la tabla vestibular como de la tabla palatina. La ALP no mostró diferencias significativas entre los grupos. El VOi fue significativamente menor en el grupo experimental con respecto a los controles. Los resultados mostraron que el modelo empleado logra una condición de osteopenia por desuso manifestada por la disminución del volumen óseo interradicular. La utilización de este modelo de desuso a diferentes tiempos experimentales permitirá evaluar las respuestas celulares de los tejidos periodontales, especialmente del tejido óseo, permitiendo por ejemplo, compararlas con respuestas conocidas como la aplicación de fuerzas ortodóncicas.

Key words: *desuso, modelo experimental, hueso alveolar.*

INTRODUCTION

Disuse osteoporosis is characterized by bone loss due to the absence of skeletal mechanical loads[1]. It occurs in situations of immobility such as paralysis associated to lesions of motor nerves or muscular dystrophy, or to changes in the mechanical environment (e.g. space flight and long-term bed rest), some of which have been taken as human models of disuse osteoporosis[2]. The most frequently cited experimental models in animals that replicate these conditions are the spinal cord injury model[3] and the hind limb unloading model[4]. Non-surgical models which have become relevant in recent years include intramuscular injection with botulinum toxin A (BTX)[5], which leads to transient muscle paralysis, resulting in a rapid loss of muscle mass and function[6,7]. All these disuse models lead to bone loss in long bones and vertebral column[5,8].

With regard to the jawbones, the model of the unopposed rodent molar has been used to study the mechanisms of tooth eruption (axial movement of teeth)[9-11]. In this model, unloading of the right-side mandibular teeth is accomplished by extraction of the right-side maxillary molars. Functional occlusion of the molars on the left side is maintained, and the time between the extractions and euthanasia has varied widely, from hours to days, weeks and even a month[12]. These studies showed that unloading-induced biological tooth movement in mice is a result of osteoclastic bone resorption on the distal aspect of the alveolar socket combined with alveolar bone and cementum formation on the mesial and apical aspects of the alveolar socket[9,10]. However, the unloading capacity in this model has not been used to evaluate potential disuse-related bone loss, which is of interest given recent use of orthodontic treatment in adults who may be suffering osteoporosis. Thus, our aim was to develop an experimental model in order to evaluate the effect of disuse on the upper jawbone in rats based on the hypothesis that the absence of teeth will lead to disuse-related bone loss in the interradicular bone of antagonist molars due to the absence of occlusal forces.

MATERIALS AND METHODS

Animal procedures

Ten male Wistar rats weighing 215-230 grams b.w. were used. All procedures were reviewed and approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires (UBACYT 2011-2014-3), which follows the Guide for the Care and Use of Laboratory Animals (NRC 1996). The animals were divided into two groups, an absolute control group (C) with n=5, and an experimental group with n=4, in which the three lower right molars were extracted, so that the zone of the upper right molars was the experimental side (E) and the zone of the upper left molars were the paired control (PC). The animals were housed in cages and fed a soft diet *ad libitum* for the duration of the experiment. Seven days after the extractions, they were euthanized by barbiturate overdose under general anesthesia.

Dental extractions

Extraction of lower molars of right hemimandible was performed under general anesthesia by intraperitoneal (IP) injection of ketamine 40 mg/kg (Ketamina 50, Holliday-Scott S.A., Beccar, Buenos Aires, Argentina) and xylazine 5 mg/kg (kensol König, Laboratorios König S.A., Avellaneda, Buenos Aires, Argentina) following the technique described by Guglielmotti and Cabrini[13]. The contralateral maxilla (left) was considered to be its paired control, and the maxillae of another five animals with all their teeth were used as absolute controls.

Sampling

The animals were sedated with 0.5 mg/kg b.w. of acepromacine and then euthanized by sodium thiopental (Pentotet®, Richmond Vet, Buenos Aires, Argentina) overdose. The jaws were positioned in occlusion, and fastened by wrapping with elastic bands to ensure contact between continuously growing incisors and molars and prevent the dental arches from separating (Fig. 1). The heads prepared in this way were resected and fixed in 10% buffered formalin.

Radiographic Techniques

After fixing, the two halves of the head were separated, while keeping the jaws closed, and standardized radiographs were taken of the jaws in occlusion (Fig. 2). Radiographs were taken using periapical dental radiographic film and MTX 70 mV 8mA dental X-ray equipment (Dental San Justo, Buenos Aires, Argentina) at 70 Kv and 0.8 sec exposure time; the focus-to-film distance

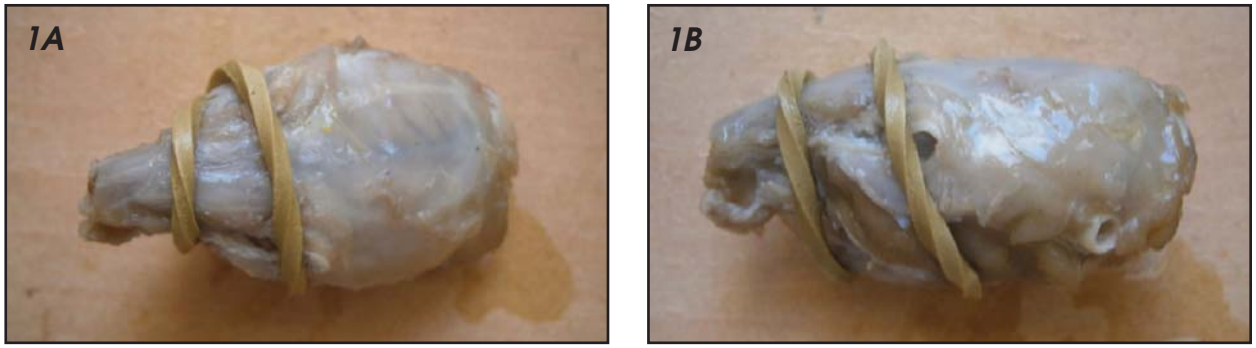

Fig. 1: Heads of animals after euthanasia, fastened in occlusion with elastic bands to ensure contact between continuously growing incisors and molars, and to prevent the separation of dental arches. A) Top view; B) lateral view.

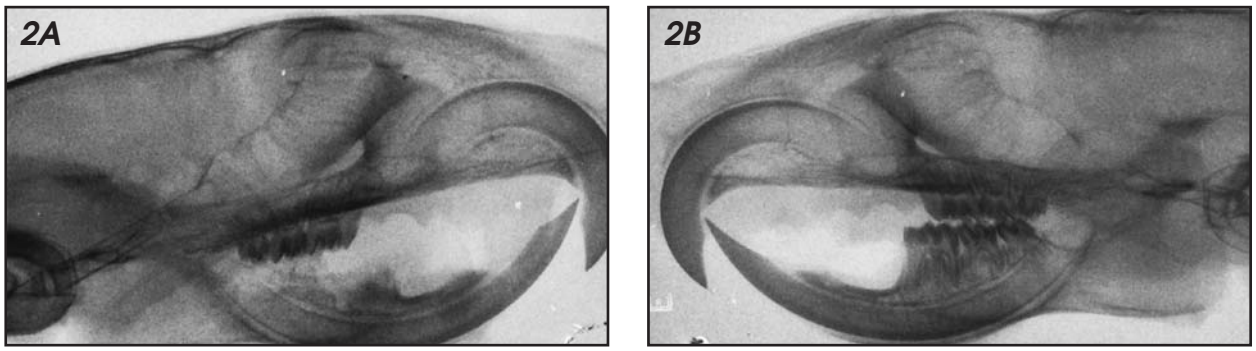

Fig. 2: Standardized radiographs of the heads with jaws in occlusion showing contact between continuously growing incisor and palatal mucosa. A) Right half of the head from the experimental group showing absence of lower molars and absence of contact between upper molars and antagonist ridge. B) Left half of the head showing molars in occlusion in an animal from which teeth were not extracted.

was 40 cm. Radiographs were processed following standard protocols. Checking contact between the continuously growing incisor and the palatal mucosa in the radiograph confirms the position in occlusion of the edentulous hemimandibles.

Sample processing

The hemimandibles were decalcified in EDTA (ethylenediaminetetraacetic acid) for four months at pH 7 and room temperature, and embedded in paraffin. Six-micron thickness bucco-palatal oriented sections at the level of the mesial root and at the level of the distal roots of the first upper molar were obtained and stained with hematoxylin-eosin (H-E) following standard protocols.

Morphometry and Histomorphometry

The radiographs were scanned with an Hp Scanjet G2710 scanner, on TMA negative scanning mode, grayscale and micrographs were taken of the histological preparations using a Canon Powershot A640 10.0 megapixel camera with 4x optical zoom (Canon Inc, Tokyo, Japan) mounted on a Carl Zeiss Axioscop 2 optical microscope (Carl Zeiss mikroscopie, Jena, Germany). The Image Pro-Plus 5.1 software was used to measure the distance from the tips of the cusps of the first upper molar to the ridge of the edentulous antagonist (DA) on the radiographs (Fig. 3A) and the following parameters on the micrographs: passive eruption degree (PED) (Fig. 3B), height of periodontal ligament (HPL) and interradicular bone volume (BV_I) (Fig. 3C). The data were compared statistically with ANOVA and Bonferroni's *post hoc* test, taking $p<0.05$ as significant.

RESULTS

Distance from the tip of the cusp of the first upper molar to the ridge of the edentulous antagonist (DA)

In the experimental group, DA showed positive values with an average of 0.34±0.048 mm, indicating that in no case was there contact between the first right upper molar and the antagonist ridge.

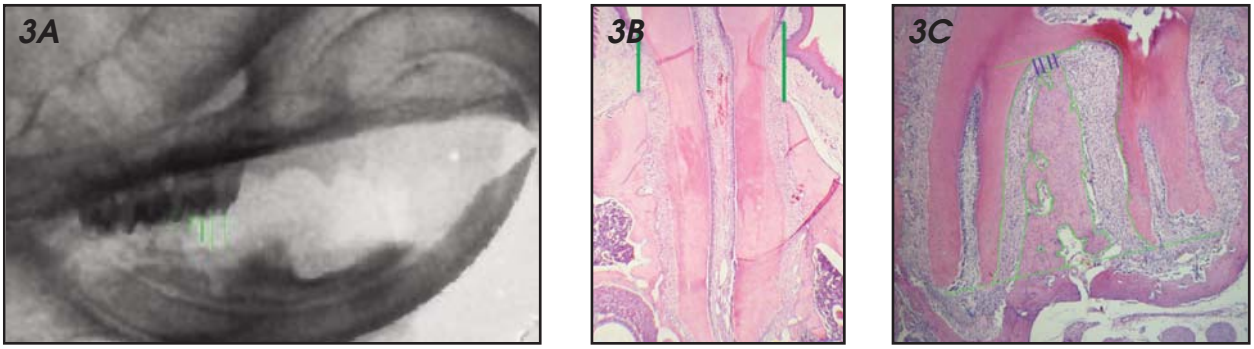

Fig. 3: Morphometry and histomorphometry measurements. A) Radiograph of one half of the head of an experimental animal showing the measurement of the distances (green lines) from the tips of the cusps of the first upper molar to the ridge of the edentulous antagonist (DA); B) Microphotograph of the mesial root of the first upper molar showing the measurement of the passive eruption degree (PED) determined as the distance from the vestibular and palatal alveolar crests to the cement-enamel junction (green lines); C) Microphotograph of distal roots of the first upper molar showing the measurement of the height of the periodontal ligament at the level of the furcation (HPL) (blue lines) and measurement of the interradicular bone volume (BVI) (green line).

Passive eruption degree (PED)

The distance from the crest of the buccal and palatal plates to the cement-enamel junction of the first upper molar was significantly greater in the experimental group than in either of the control groups and on both plates, with no difference between the paired control group (PC) and the absolute control group (C) (Table 1).

Height of the periodontal ligament (HPL)

There was no significant difference in the height of the periodontal ligament among the 3 groups (Table 2).

Interradicular bone volume (BV/TV)

Interradicular bone volume was significantly lower in the experimental group than in the control groups, and there was no difference between control groups (Table 2).

DISCUSSION

Although the literature includes studies in which occlusal forces are eliminated, this is the first study to conduct a histomorphometric evaluation of the antagonist alveolar bone to the edentulous ridge[9-11]. It is important to note that most experimental orthodontic models used universally are applied on the first upper molar. Thus, having a model that replicates a disuse condition in the upper jaw allows us to compare conditions with and without forces on the same bone.

Disuse osteoporosis corresponds to bone loss due to skeletal mechanical unloading. The condition has been observed in various situations, and can be described as models with immobilizations linked to changes in mechanical environment, such as space flight or long term bed-rest, and pathological immobilizations, such as those observed during neurological or muscular diseases, spinal cord injury being the most frequently quoted model[1,2]. The notion of disuse osteoporosis appeared in the literature in the seventies with the reports of histomorphometry analysis of iliac crest bone biopsies performed at various times after the spinal cord injury[14]. Trabecular bone volume exhibits rapid bone loss in the first three

Table 1: Degree of passive eruption (DE). Distance from buccal bone crests (BP) and palatal bone crest (PP) to the cementoenamel junction.

	BP	PP
E	523.55 ± 5.68 µm*	949.83 ± 136.85 µm*
C	251.73 ± 53.61 µm	427.51 ± 142.53 µm
PC	295.54 ± 82.85 µm	509.30 ± 58.96 µm

E: experimental side; C: absolute control;
PC: paired control side; *p<0.05

Table 2: Height of the periodontal ligament (HPL) measured at the level of the furcation and interradicular bone vloume (BV/TV).

	E	PC	C
HPL	124.5±11.99µm	163.76 ±91.96µm	156.11±78.26µm
BV/TV	0.78±0.12*	0.88±0.03	0.82±0.06

E: experimental side; C: absolute control
PC: paired control side; *p<0.05

months of disuse, followed by slower loss. In parallel, bone remodeling is found uncoupled with an increase in bone resorption and a decrease in static and dynamic bone formation parameters[15].

The use of this model with different experimental times will enable an evaluation of cell responses in periodontal tissues, particularly bone tissue, which can be compared to the known response of the bone to the application of forces such as orthodontic forces, and in various systemic conditions. In addition, it will subsequently allow us to compare the results with other models of absence of force in other parts of the body (tibia, femur, spine), and to compare them to the external application of forces or of different systemic conditions. The fact that there was no significant difference in the results between the Control group and the Paired Control group means that fewer experimental animals will be needed in further studies.

Potential results of further experiments replicating the disuse condition in the upper maxillary bone due to absence of occlusal forces caused by missing antagonist teeth will be transferred to the clinical understanding of the biological mechanisms governing periodontal tissues and alveolar bone of partially edentulous patients who do not use dental prostheses to replace their missing teeth.

The frequency of aged patients receiving orthodontic treatment has increased in recent years. Osteoporosis is often associated with aging. Recent studies[16,17] report that ovariectomy (OVX) accelerated orthodontic tooth movement (OTM) in rats. Acceleration of OTM is expedient for orthodontists because it can reduce treatment duration; however, it also involves the risk of side effects. These studies showed that OVX accelerates OTM but also induces severe root resorption. With the increasing demand for orthodontic treatment in adult patients, orthodontists need up-to-date knowledge regarding age-related metabolic changes and the effects of medications.

The reduction in bone volume measured after applying the proposed model shows that it is effective for inducing disuse oestopenia in the alveolar bone in the jaws of rats.

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