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Histological Evaluation of the Effect of Alendronate on the Maxillary Bone of Wistar Rats Combined with Orthodontic Movement of Intense Force

Evaluación Histológica del Efecto del Alendronate en el Hueso Maxilar de Ratas Wistar Combinado con Movimiento de Ortodoncia de Fuerzas Intensas

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SUMMARY: To evaluate the histological adverse effects of alendronate administered systemically and topically in combination with orthodontic movement by intense force. Thirty-six 24-week-old female Wistar rats, ovariectomized, were used and divided into three groups (n = 12/group): control, locally treated with saline (0.07 ml/kg/week) (group 1) and experimental, treated with alendronic acid systemically (0.07 mg/kg/week) (group 2) and locally (7 mg/kg/week) (group 3). At 14 days, an orthodontic anchor was installed in the right first molar, and a force of 144 cN was applied for 28 days. The samples were processed for histological evaluation. Descriptive statistics, Shapiro-Wilk tests, one-way ANOVA with Bonferroni correction, one-way repeated measures ANOVA and chi-square tests were performed. All tests were statistically significant at $p < 0.05$. The adverse events found in all groups were inflammation and osteoclastic activity. In the bisphosphonate-treated groups, there were statistically significant differences ($p = 0.005$) in the osteoclastic activity between the two hemiarcates. All rats in group 2 presented paralytic ileus. Compared to local administration, systemic treatment with alendronic acid produces more adverse effects, such as inflammation, fibrinoid necrosis, and osteoclastic activity. During the application of intense forces, it was not possible to show that there is necrosis associated with bisphosphonates.

KEY WORDS: Histology; Bone biology; Anchorage; Appliances.

INTRODUCTION

Orthodontic treatment has become a more accessible procedure and one that is increasingly performed in adults. Currently, orthodontists have increased the probability of treating patients with a medical history related to diseases of the skeletal system, such as osteoporosis (Salazar *et al*., 2015), multiple myeloma, bone metastases, hypercalcemia, or Paget's disease (Zahrowski, 2007).

During the controlled application of mechanical forces in orthodontic movement, an alteration in blood flow takes place on the pressure side where a great variety of regulatory factors are generated, and cells such as osteoblasts, osteoclasts, and osteocytes, all of which are involved in bone remodeling between the periodontal ligament and the alveolar bone, are activated (Kaipatur *et al*., 2013; Nakasˇ *et al*., 2017). For this reason, orthodontists must know the effects of drugs that can condition dental movement.

Bisphosphonates (BPs) are drugs indicated for the treatment of osteopenic and osteoporotic states (Salazar *et al*., 2015). Their activity is based on the inhibition of reabsorption in bone mineralization, interfering with osteoclastic activity (Zahrowski, 2007).

Topical or systemic application of bisphosphonates may have side effects in dental treatment. In the field of orthodontics, some of the effects may be beneficial for treatment since they reduce the recurrence of dental movement, skeletal recurrence after maxillary expansion, or mandibular distraction (Iglesias-Linares *et al*., 2010) and

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can act as a pharmacological anchor modulating dental movement (Karras *et al*., 2009).

However, it is essential to consider bisphosphonate type-dependent adverse effects, dose, duration of treatment, and administration (topical, oral or intravenous). These can cause impaired bone scarring (Zahrowski, 2007) or osteonecrosis of the jaws (Zahrowski, 2007).

There are different types of bisphosphonates, such as alendronate (Zahrowski, 2007; Karras *et al*., 2009; Salazar *et al*., 2015; Franzoni *et al*., 2017), etidronate (Zahrowski, 2007), pamidronate (Kim *et al*., 1999; Zahrowski, 2007; Venkataramana *et al*., 2012), risedronate (Wu *et al*., 2019), tiludronate (Zahrowski, 2007), zoledronic acid (Zahrowski, 2007; Helm *et al*., 2010), and clodronate (Choi *et al*., 2010).

Sodium amino bisphosphonate alendronate (ALN) is one of the most widely used bisphosphonates for the treatment of osteoporosis or osteopenia due to its high potential to inhibit bone resorption (Karras *et al*., 2009; Salazar *et al*., 2015).

The most commonly used animal model for assessing the effects of alendronate on the application of mechanical forces during orthodontic anchorage has been rats (Igarashi *et al*., 1994; Karras *et al*., 2009; Kaipatur *et al*., 2013; Komatsu *et al*., 2013; Salazar *et al*., 2015; Franzoni *et al*., 2017). Different alendronate doses or follow-up times have been evaluated in these studies. However, only one study (Igarashi *et al*., 1994) has analyzed the effect of alendronate at the histological level, varying the route of administration (topical or systemic), and no research has investigated the application of excessive force to assess bone necrosis.

This aim of this study was to evaluate the histological adverse effects of alendronate administered systemically and topically in combination with orthodontic movement by intense force.

MATERIAL AND METHOD

The recommendations published in the ARRIVE (Kilkenny *et al*., 2010) (Animal Research: Reporting of In Vivo Experiments) guidelines were followed in this study.

All surgical and experimental procedures were approved by the Animal Experimentation Ethics Committee of the University of Zaragoza (Ref. PI 22/13), complying with the ethical principles and protection of animals used for experimentation. This study was carried out under the regulations regarding the protection of animals used for scientific purposes (Law 32/2007) (BOE-A-2015-3564 Orden ECC/566/2015, de 20 de marzo, por la que se establecen los requisitos de capacitación que debe cumplir el personal que maneje animales utilizados, criados o suministrados con fines de experimentación y otros fines científicos, incluyendo la docencia) (Boletín Oficial del Estado, 2015). All surgery was performed under sodium pentobarbital anesthesia and every effort was made to minimize suffering.

In this clinical trial, 36 female 24-week-old Wistar Han rats, weighing approximately 300 g, with proven reproductive capacity were used. They were obtained from Harland Farm Inc. (Envigo, Indianapolis, IN), authorized and registered for the breeding of experimental animals. Ovariectomy was performed in all the animals before the division of the groups.

Rats were housed under conventional conditions in polycarbonate type IV cages in groups of three at constant temperature (20±4°C) and humidity (41 %) under a 12-hour light/dark cycle with free access to ration and water. The animals underwent an adaptation period of 7 days from their arrival at the Research Center until starting the experimental procedure.

The size of the sample was determined taking into account the studies published in rats on the evaluation of movement with orthodontics and its effect due to the administration of bisphosphonates. The most extensive sample size studies were those of Karras *et al*. (2009) and Salazar *et al*. (2015), with 12 rats in each group. Therefore, in our study, we used 36 rats divided into three groups.

The animals were divided by a list of random numbers into three groups, one control and two experimental groups, with the administration of alendronic acid (Fosamax®; Merck & Co, Inc, Whitehouse Station, NJ) by systemic and local administration routes (Fig. 1) as follows:

Group 1 (n = 12): control group with subcutaneous administration in the lower vestibule in the right upper first molar with saline solution (0.07 ml/kg/week) for 14 days, installation of anchorage and orthodontic movement, and follow-up for an additional 14 days.

Group 2 (n = 12): experimental group with systemic administration of alendronic acid (7 mg/kg/week) by intraperitoneal injection for 14 days, installation of anchorage and orthodontic movement, and follow-up for an additional 14 days.

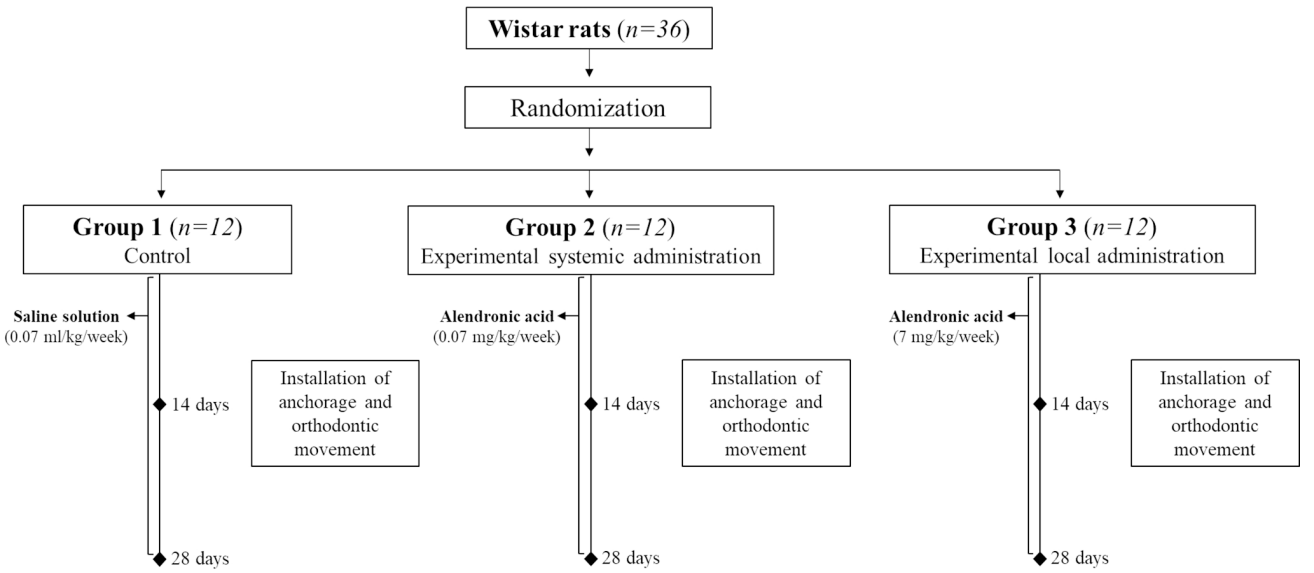

Fig. 1. Study design.

Group 3 (n = 12): experimental group with local administration of alendronic acid (0.07 mg/ml/week) by injection into the vestibule at the right upper first molar for 14 days, installation of anchorage and orthodontic movement, and follow-up for an additional 14 days.

The weight of the animals was collected at the beginning of the study and weekly until the end of the study.

The animals were sedated by subcutaneous injection in the corridor at the level of the right first molar with sevoflurane (3.5 % + O2) for the administration of the medications. For the placement of the orthodontic appliances, they were injected intraperitoneally with a combination of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg).

The placement of the orthodontic anchor was always performed by the same operator. A 6 mm long nickel-titanium closed spring with a force of 147 gr was used between the ligatures placed between the right upper first molar and the upper incisors. The ligatures were fixed to the teeth employing acid conditioning, adhesive, and light-curing resin (Transbond™ Plus Self-Etching Primer, 3M, USA, CA) (Fig. 2).

During the postoperative period, the animals were checked daily, including the physical appearance of the animal and the orthodontic device.

All animals were euthanized at 28 days by anesthetic induction and intracardiac injection of sodium thiopental. The jaws were removed and fixed in 10 % formalin.

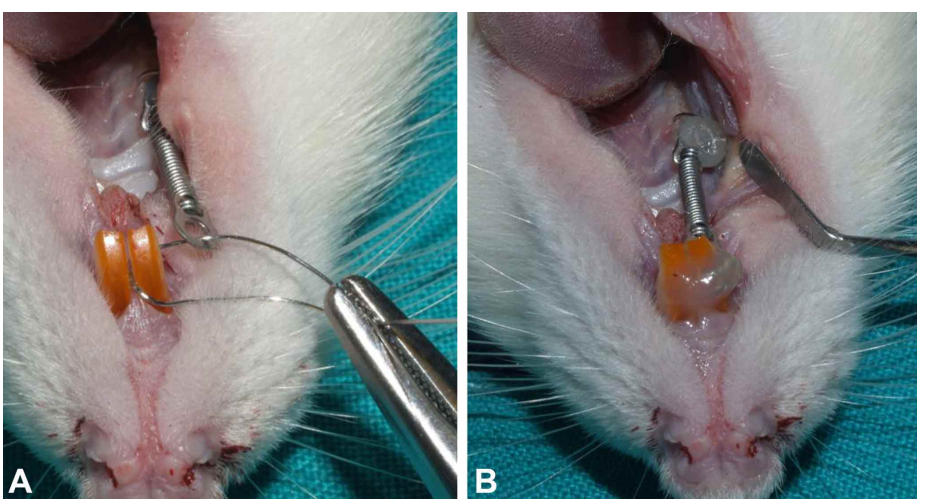

Fig. 2. Orthodontic anchoring procedure. a) Fixation of the metallic ligature to the incisors. b) Visualization of the final device.

Subsequently, they were isolated and decalcified with a 5 % nitric acid solution for 48 hours. The maxilla was divided into two hemiarcates, which were processed using a standard histological technique and embedded in paraffin.

Histological sections were cut at 4 microns and stained with a hematoxylin and eosin solution.

A Nikon® Eclipse Ci (Nikon Instruments Inc., USA, NY) microscope was used, and photographs were taken with a Nikon® DS-Fi3 digital system.

HISTOLOGICAL ANALYSIS EVALUATION

The following variables were evaluated by a pathology expert using a 40X light microscope.

Bone neoformation: 0: Osteoid proliferation is nonexistent; 1: Very slight when the bone neoformation has between 2 and 4 osteocytes; 2: mild, when the new formation has between 4 and 6 osteocytes; 3: moderate, bone callus with the presence of 6 to 8 osteocytes; 4: intense, when the bone neoformation has between 8 and 10 osteocytes.

Osteoclastic activity: 0: when there is no osteoclastic activity; 1: very slight, activity is between 2 and 4 osteoclasts; 2: mild, activity is between 4 and 6 osteoclasts; 3 moderate, osteoclastic activity between 6 and 8; 4: intense, osteoclastic activity is between 8 and 10.

Inflammation intensity: 0 the absence of infiltration of mononuclear cells (macrophages, lymphocytes, and plasma cells); tissue destruction from chronic inflammation; reconstruction attempt by fibrosis and angiogenesis. A score of 1 was established with the presence of all the previously described factors being very slight; 2 mild presence of mononuclear cell infiltration (macrophages, lymphocytes, and plasma cells); tissue destruction from chronic inflammation; reconstruction attempt by fibrosis and angiogenesis; 3 moderate presence and 4 intense inflammation.

Fibrinoid necrosis: 0, when there is no presence of biological components stained with hematoxylin-eosin, 1 very mild inflammatory component and staining, 2 mild, 3 moderate inflammatory component and moderate staining of eosinophilic areas and 4 intense activity of the inflammatory component and intense staining.

Foreign body: very large, 0.5 mm in size, (score = 4); moderate, with a size of 0.4 mm (score = 3); small, with a size of 0.3 mm (score = 2); very small, with a size of 0.15 mm (score = 1); and nonexistent foreign body with a value of 0.

Paralytic ileus: 0 absence, 1 presence.

Statistical analysis. Descriptive statistics of continuous quantitative variables (mean and standard deviation) and ordinal qualitative variables (frequency and percentage) were performed. Normality of the distribution was analyzed using the Shapiro-Wilk test. One-way ANOVA with Bonferroni correction was used in the statistical analysis to compare the weight between the groups, and to compare the progress of each group weekly, one-way repeated measures ANOVA was used. The chi-square test was used in the statistical analysis of the variables evaluated histologically. The list of the number of rats randomized to distribute the 36 rats in the three study groups was generated from seed 23. All statistical tests were performed at a 95 % confidence level with Stata 11.1 software (Stata Corp, College Station, TX, USA).

RESULTS

Study follow-up was completed with 36 rats, but two of the animal samples were rendered useless for histological analysis due to microtome technical problems—one in the control group and the other in the systemic bisphosphonate group. The average time for the orthodontic anchoring procedure was 20.46 minutes.

Table I shows the weights of the rats (mean and standard deviation) in all groups. During the study, there were significant differences between the weights of the groups treated with bisphosphonates ($p < 0.05$). In general, there was a weight change in all groups, and from 14 days onward, the trend was towards a decrease.

Table I. Weight of rats per group during study period.

	Baseline	SD	7 days	SD	14 days	SD	21 days	SD	28 days	SD
Group 1	299.33	21.24	300.50	18.11	277.17	17.12	271.67	26.03	264.08	25.98
Group 2	268.17*	46.46	270.75*	45.86	256.17*	44.61	246.25*	44.13	241.00*	51.77
Group 3	309.00*	40.62	315.00*	41.49	295.25*	37.85	280.58*	35.61	281.17*	36.27

SD: Standard deviation. * Significant results ($p < 0.05$) in one factor ANOVA + post hoc Bonferroni. There were no significant results for one-way repeated measures ANOVA.

Histological analysis. There was more bone neoformation in the control group on the right side (45.45 %). On the left side, bone development was not observed in any of the groups. There were no significant differences between the two sides (Table II).

Inflammation occurred in all groups, and there were no significant differences between the groups or between hemiarches. Inflammation on the right side was more predominant, occurring in more than 90 % of the rats (Table III).

More considerable fibrinoid necrosis was observed in the group of systemic bisphosphonates (72.73 % right hemiarch, 27.27 % left hemiarch) (Fig. 3). No significant differences were observed between the groups or between the two sides (Table IV).

Table II. Bone neoformation on both sides of the maxilla in the study groups.

	Bone neoformation R						Bone neoformation L					
	Group 1		Group 2		Group 3		Group 1		Group 2		Group 3	
Grade	F	%	F	%	F	%	F	%	F	%	F	%
Absence	6	54.55	10	90.91	10	83.33	11	100	11	100	12	100
Very slight	1	9.09	0	0.00	1	8.33	0	0.00	0	0.00	0	0.00
Slight	2	18.18	0	0.00	1	8.33	0	0.00	0	0.00	0	0.00
Moderate	2	18.18	1	9.09	0	0.00	0	0.00	0	0.00	0	0.00
Intense	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Total	11	100	11	100	12	100	11	100	11	100	12	100

R: Right hemiarch; L: Left hemiarch; F: Absolute frequency; %: percentage. There were no significant results in the chi-square test.

Table III. Inflammation on both sides of the maxilla in the study groups.

	Inflammation R						Inflammation L					
	Group 1		Group 2		Group 3		Group 1		Group 2		Group 3	
Grade	F	%	F	%	F	%	F	%	F	%	F	%
Absence	1	9.09	0	0.00	0	0.00	7	63.64	2	18.18	1	8.33
Very Slight	5	45.45	3	27.27	4	33.33	2	18.18	6	54.55	7	58.33
Slight	2	18.18	5	45.45	6	50.00	2	18.18	2	18.18	4	33.33
Moderate	2	18.18	2	18.18	1	8.33	0	0.00	1	9.09	0	0.00
Intense	1	9.09	1	9.09	1	8.33	0	0.00	0	0.00	0	0.00
Total	11	100	11	100	12	100	11	100	11	100	12	100

R: Right hemiarch; L: Left hemiarch; F: Absolute frequency; %: percentage. There were no significant results in the chi-square test.

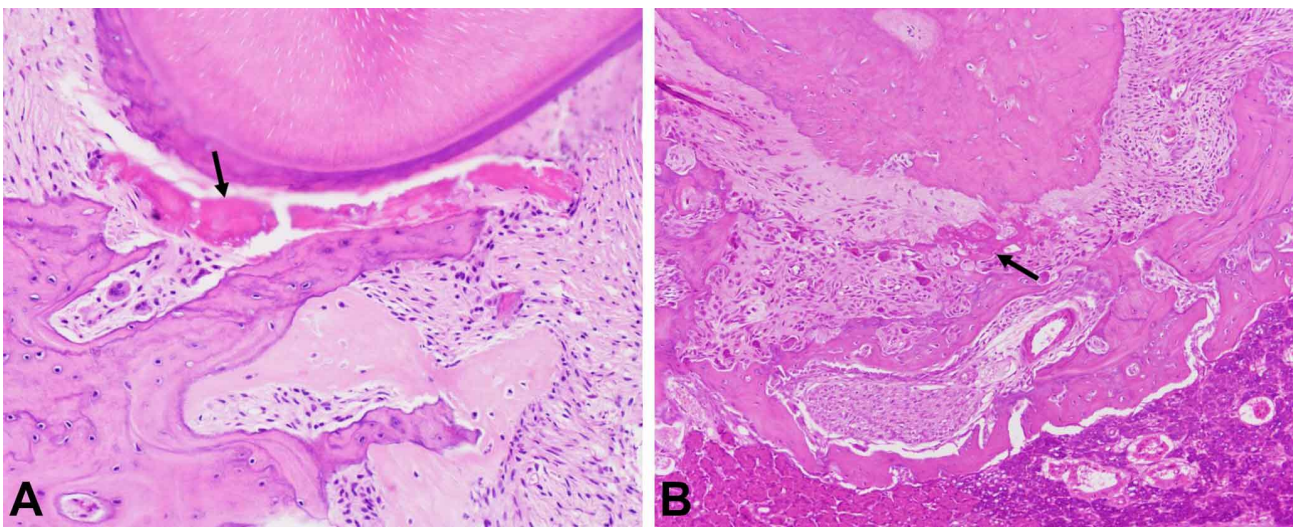

Fig. 3. Fibrinoid necrosis and osteoclastic activity (indicated with the arrow). HE x100. a) In the systemic administration group at the base of the molar. b) In the local administration group in the periodontal ligament.

Table IV. Fibrinoid necrosis on both sides of the maxilla in the study groups.

	Fibrinoid necrosis R						Fibrinoid necrosis L					
	Group 1		Group 2		Group 3		Group 1		Group 2		Group 3	
Grade	F	%	F	%	F	%	F	%	F	%	F	%
Absence	6	54.55	3	27.27	7	58.33	10	90.91	8	72.73	10	83.33
Very Slight	3	27.27	0	0.00	3	25	0	0.00	2	18.18	2	16.67
Slight	0	0.00	6	54.55	2	16.67	1	9.09	1	9.09	0	0.00
Moderate	1	9.09	1	9.09	0	0.00	0	0.00	0	0.00	0	0.00
Intense	1	9.09	1	9.09	0	0.00	0	0.00	0	0.00	0	0.00
Total	11	100	11	100	12	100	11	100	11	100	12	100

R: Right hemiarch; L: Left hemiarch; F: Absolute frequency; %: percentage. There were no significant results in the chi-square test.

The presence of foreign bodies (plant material and hairs) embedded in the gums of the animals was studied, causing adverse events from inflammation to purulent gingivitis. In the local bisphosphonate group, a higher percentage of foreign bodies was observed on both sides. No significant differences were observed between the groups or between the two sides (Table V).

All rats showed osteoclastic activity in the right hemiarch. In the left hemiarch in the groups treated with bisphosphonates, systemic and topical, there was a higher total percentage of osteoclastic activity, 100 % and 91.67 %, respectively. Significant differences were shown on this side ($p = 0.005$) (Table VI).

At necropsy, paralytic ileus was observed in the entire group treated with systemic bisphosphonates. These differences were significant ($p = 0.000$) between the control group and the group treated with topical bisphosphonates where paralytic ileus did not occur (Table VII) (Fig. 4).

Adverse events. In the control group, the most frequent adverse events were inflammation in the right hemiarch (90.9 %) and osteoclastic activity (right hemiarch 100 %, left hemiarch 72.73 %).

In the group treated with systemic bisphosphonates, inflammation (100 % right hemiarcade, 81.82 % left hemiarcade), fibrinoid necrosis (72.73 % right hemiarcade), osteoclastic activity (100 % on both sides) and paralytic ileus were observed in all rats in the group.

In the group treated with local bisphosphonates, inflammation (100 % right hemiarch, 91.66 % left hemiarch) and osteoclastic activity (100 % right hemiarch, 91.67 % left hemiarch) were observed.

Table V. Presence of foreign body on both sides of the maxilla in the study groups.

	Foreign body R						Foreign body L					
	Group 1		Group 2		Group 3*		Group 1		Group 2		Group 3*	
Grade	F	%	F	%	F	%	F	%	F	%	F	%
Absence	8	72.73	9	81.82	8	66.67	10	90.91	9	81.82	8	66.67
Very small	0	0.00	0	0.00	1	8.33	0	0.00	0	0.00	0	0.00
Small	1	9.09	2	18.18	2	16.67	1	9.09	1	9.09	4	33.33
Moderate	2	18.18	0	0.00	1	8.33	0	0.00	1	9.09	0	0.00
Large	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Total	11	100	11	100	12	100	11	100	11	100	12	100

R: Right hemiarch; L: Left hemiarch; F: Absolute frequency; %: percentage. * Significant results ($p < 0.05$) in the chi-square test.

Table VI. Osteoclast activity on both sides of the maxilla in the study groups.

	Osteoclast activity R						Osteoclast activity L					
	Group 1		Group 2		Group 3		Group 1*		Group 2*		Group 3*	
Grade	F	%	F	%	F	%	F	%	F	%	F	%
Absence	0	0.00	0	0.00	0	0.00	3	27.27	0	0.00	1	8.33
Very Slight	0	0.00	0	0.00	0	0.00	6	54.55	10	90.91	3	25
Slight	1	9.09	6	54.55	5	41.67	2	18.18	1	9.09	8	66.67
Moderate	9	81.82	5	45.45	5	41.67	0	0.00	0	0.00	0	0.00
Intense	1	9.09	0	0.00	2	16.67	0	0.00	0	0.00	0	0.00
Total	11	100	11	100	12	100	11	100	11	100	12	100

R: Right hemiarch; L: Left hemiarch; F: Absolute frequency; %: percentage. * Significant results ($p < 0.05$) in the chi-square test..

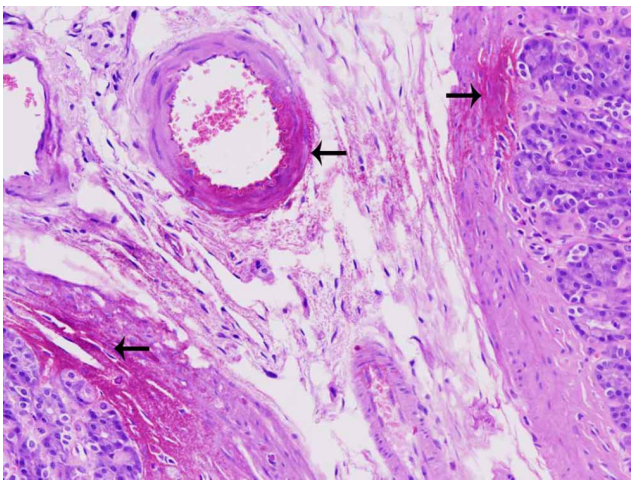

Fig. 4. Paralytic ileus from a histological sample of a rat from the systemically treated alendronate group (indicated with the arrow). Calcification is observed in the wall of an artery between the connective tissue of the submucosa and the glands of the intestinal mucosa. HE x 200.

Table VII. Presence of paralytic ileus at necropsy in the study groups.

	Paralytic ileus					
	Group 1*		Group 2*		Group 3*	
Grade	F	%	F	%	F	%
No	12	100	0	0.00	12	100
Yes	0	0.00	12	100	0	0.00
Total	12	100	12	100	12	100

F: Absolute frequency; %: percentage. * Significant results ($p < 0.05$) in the chi-square test.

DISCUSSION

Oral alendronate is one of the most prescribed bisphosphonates in Spain, being one of the least expensive compared to the rest of the recommended bisphosphonates. The bisphosphonates interrupt the cellular balance of the bone cells, causing the destruction of bone vascularization and reducing the movement of the teeth with orthodontic anchorage. There are numerous studies in animal models where the administration of bisphosphonates affects the movement of the tooth (Kaipatur *et al*., 2013; Salazar *et al*., 2015; Fernández-González *et al*., 2016), and positive effects can be found for its control during orthodontic treatment or to prevent recurrences (Karras *et al*., 2009; Iglesias-Linares *et al*., 2010). However, the findings of our study are aimed at the qualitative evaluation of the cells involved in the balance of bone remodeling under the condition of application of inadequate force.

This study's results reveal more significant osteoclastic activity in the groups treated with alendronic acid and specifically administered systemically. These findings differ from Franzoni *et al*. (2017), where ovariectomies were not performed and less osteoclastic activity was found.

Incorporation of ovarian removal surgery into the design emulates bone loss due to estrogen depletion, creating a situation similar to osteoporosis caused by menopause. We have incorporated the simulation of osteoporotic bone in our study, and nevertheless, we have found results opposite to those of Salazar *et al*. (2015), where the administration of alendronic acid in ovariectomized rats reduces osteoclastic activity.

All these differences found may be due to the application of excessive force (144 cN) in orthodontic movement compared to other designs under bisphosphonate administration conditions in rats where the force oscillates between 10 cN (Ren *et al*., 2004) and 50 cN (Salazar *et al*., 2015). Most of the studies on the effects of orthodontic movement carried out in this animal model do not exceed 100 cN (Kondo *et al*., 2017; Alhasyimi & Rosyida, 2019; Kaya *et al*., 2020). Pereira *et al*. (2010) assessed the histological effects of induced subluxation in rat molars and their implications in orthodontic treatment by applying forces between 600 and 1000 cN. However, in the literature, we have not found any study that evaluated the histological results of the application of excessive forces in orthodontic movement under conditions of bisphosphonate administration.

Despite the randomization of the rats in the distribution of the groups, there were significant differences between the weights of the rats in the two experimental groups. However, follow-up throughout the study did not reveal significant differences. Although ovariectomy was performed in all rats, there was no weight gain during the study. These findings coincide with those found in other studies (Lee *et al*., 2001; Kohno *et al*., 2002; Kaipatur *et al*., 2013). Our design may be due to excessive inflammation, which does not allow rats to feed appropriately.

The effect of bisphosphonates has been studied in humans and animal models such as rabbits (Venkataramana *et al*., 2014) or rats (Kaipatur *et al*., 2013; Salazar *et al*., 2015). The latter has been used very frequently. In our study, we found paralytic ileus in all rats in the systemic alendronate group. These findings suggest that drug administration routes other than peritoneal injection, such as tail vein injection, should be considered to avoid these undesirable conditions. Although we have not shown that there is bone necrosis due

to excessive force during orthodontic treatment under the administration of bisphosphonates, we cannot rule out its eventual occurrence if the study time had been longer. The number of adult patients taking bisphosphonates has increased, and despite human and animal studies, it is advisable to assess the long-term effects of high intensity levels on applied forces.

CONCLUSION

Compared to local administration, systemic treatment with alendronic acid produces more adverse effects, such as inflammation, fibrinoid necrosis, and osteoclastic activity. During the application of intense forces, it has not been possible to show that there is necrosis associated with bisphosphonates.

CINTORA, L. P.; ROJO, R.; PASTOR, O. C. & WHITE, A. Evaluación histológica del efecto del alendronate en el hueso maxilar de ratas wistar combinado con movimiento de ortodoncia de fuerzas intensas. *Int. J. Morphol., 41(3):*901-909, 2023.

RESUMEN: Evaluar los efectos adversos histológicos del alendronato administrado sistémica y tópicamente en combinación con movimientos ortodóncicos de fuerza intensa. Treinta y seis ratas Wistar hembras de 24 semanas de edad, ovariectomizadas, fueron utilizadas y divididas en tres grupos (n = 12/grupo): control, tratado localmente con solución salina (0,07 ml/kg/semana) (grupo 1) y experimental, tratados con ácido alendrónico por vía sistémica (0,07 mg/kg/semana) (grupo 2) y local (7 mg/kg/semana) (grupo 3). A los 14 días se instaló un anclaje de ortodoncia en el primer molar derecho y se aplicó una fuerza de 144 cN durante 28 días. Las muestras fueron procesadas para evaluación histológica. Se realizó estadística descriptiva, pruebas de Shapiro-Wilk, ANOVA de una vía con corrección de Bonferroni, ANOVA de medidas repetidas de una vía y pruebas de chi-cuadrado. Todas las pruebas fueron estadísticamente significativas con un $p < 0,05$. Los eventos adversos encontrados en todos los grupos fueron inflamación y actividad osteoclástica. En los grupos tratados con bisfosfonatos hubo diferencias estadísticamente significativas ($p = 0,005$) en la actividad osteoclástica entre los dos hemiarcados. Todas las ratas del grupo 2 presentaron íleo paralítico. En comparación con la administración local, el tratamiento sistémico con ácido alendrónico produce más efectos adversos, como inflamación, necrosis fibrinoide y actividad osteoclástica. Durante la aplicación de fuerzas intensas, no fue posible demostrar que existe necrosis asociada con los bisfosfonatos.

PALABRAS CLAVE: Histología; Biología ósea; Anclaje; Accesorios.

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