

Effect of Growth Hormone in Experimental Tooth Movement

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The aim of this study was to evaluate, by histological analysis, the effect of growth hormone (GH) on periodontal ligament and alveolar bone during experimental tooth movement in rats. Eighty male Wistar rats divided into control (C) and experimental (E) groups were examined after 3, 7, 14 and 21 days under controlled climate conditions. Orthodontic force (30 cN) was applied on the maxillary first molar by an orthodontic appliance. Group E received 0.1 IU/kg/day of GH and Group C received 0.5 mL/kg/day of saline. The samples were processed and evaluated under optical microscopy and polarized light microscopy. The Kruskal Wallis test was applied to compare the intergroup variables at 5% significance level. Group E presented a larger number of osteoclasts on the 3rd and 7th days and Howship lacunae on the 3rd day, a smaller number of blood vessels and greater amount of mature collagen on the 3rd and 7th days than Group C ($p < 0.05$). It was concluded that GH accelerated and intensified bone resorption and produced delay in immature collagen formation during experimental tooth movement.

Introduction

Growth hormone (GH) is an important and powerful metabolic hormone that is secreted from cells in the anterior pituitary gland, influenced by several normal and pathophysiological conditions. The molecular structure consists of a single polypeptide chain of 191 amino acids with two disulphide bridges and a molecular weight of 22 kDa (1) and has a plasmatic half-life between 15 and 20 min after secretion or intravenous injection. After subcutaneous or intramuscular administration, blood concentrations of GH reach a peak between 1 and 3 h after injection and fall to undetectable levels after 24 h (2).

GH is one of the most important regulatory substances in bone growth and bone remodeling (3). Children with various forms of GH deficiency or other defects in the GH signal-transduction pathway typically do not reach their full potential of size and stature (4). Until recently, GH supply was too much limited to treat these patients, but the advent of commercial application of recombinant DNA technology increased the indication of treatment with systemic supply of GH (5). Currently, individuals with specific endocrinopathies, debilitations associated with AIDS, Turner syndrome, chronic kidney failure (6), chronic obstructive lung disease (7) and sepsis (8) use synthetic GH in their treatments.

This hormone is also used illicitly and without medical supervision to improve the performance of athletes and people training at gyms. One study has reported that 5% of male American high-school students use or have used GH as an anabolic agent (1). Furthermore, GH may revert

some changes associated with aging, which is associated with the reduction in the blood levels of this hormone, and its administration can cause increase in density and skin collagen and/or increase lean body mass, muscular mass and bone mass (9,10).

Considering its wide indication and use as well as the consolidation of its anabolic and lipolytic effects, and its direct action on bone formation and resorption (11-13), one could question whether GH would have the competence to interfere in bone remodeling during experimental tooth movement. GH acts directly on the proliferation and differentiation of osteoblasts (3) thus stimulating bone turnover, causing the increase in protein synthesis and mineralization (14) and more specifically, the bone matrix proteins (3). However, there are no studies in the literature demonstrating whether there is any modification in bone remodeling induced by orthodontic treatment in individuals on GH supplementation.

Bone formation requires differentiated and active osteoblasts to synthesize the extracellular matrix that will support the mineralizing process (15). Orthodontic tooth movement produces tissue reactions that are associated not only with local factors related to teeth and occlusion, but also with systemic factors related to bone metabolism (16,17).

The aims of this study were to verify the effect of GH by the quantification of osteoclasts, Howship lacunae and blood vessels in the periodontal ligament and by the analysis of collagen maturation during experimental tooth movement in Wistar rats.

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Material and Methods

The research protocol was approved by the institutional Ethics Committee for Animal Experimentation (Process #395).

Eighty 12-week-old male Wistar rats (*Rattus norvegicus albinus*) weighing approximately 300–350 g from our institution's experimental animal care facility were used. The animals were kept in plastic cages, between 19 °C and 22 °C and photoperiod of 12 h of light and 12 h of darkness to avoid alteration in the metabolic cycle, with water and food *ad libitum*.

The animals were randomly divided into 2 groups: control (CG) and experimental (EG). Group C received saline at 0.5 mL/kg daily to simulate stress suffered by the animals up to the moment of euthanasia after 3, 7, 14 and 21 days. Group E received 1.33 mg (4 IU) of Saizen® growth hormone (Somatropina r-hGH; Lab. Serono, Aubonne, Switzerland). Daily applications of 0.1 IU/kg was administered subcutaneously in the abdominal area alternating between the right and left side, beginning one day before orthodontic appliance installation and continuing up to the moment of euthanasia, always at the same time of the day. To receive the appliance, the animals were sedated with an intramuscular injection of tiletamine hydrochloride: zolazepam hydrochloride (Zoletil 50; Virbac do Brazil Ind. e Com., São Paulo, SP, Brazil; 50 mg/kg body wt; mean volume 0.25 mL/animal) in the quadriceps muscle of the left lower limb.

The orthodontic appliance (Fig. 1) was made from the model proposed by Heller and Nanda (18), which consisted of a 9 mm closed nickel-titanium spring (G&H® Wire Company, Hannover, Germany) and a 0.022 inch stainless steel wire tie to fix the spring to the first right molar and maxillary incisors, with a force of 30 cN, measured by a precision dynamometer (Dentaurum, Ispringen, Germany). After initial activation, the appliance was not reactivated during the experimental period, but its position was checked daily.

The animals were euthanized by an intraperitoneal overdose of anesthetic solution, the anatomic pieces containing the maxillary right first molar were dissected, removed, fixed in 10% buffered formalin for 72 h and demineralized in a 4.13% EDTA solution for 12 weeks. Next, After demineralization, 4-µm-thick cross-sectional serial sections were obtained from the alveolar bone crest up to the root apex and stained with Harris and Lison hematoxylin-eosin or picosirius method for analysis by a single, calibrated operator blinded to the groups.

The hematoxylin-eosin method was used to recognize and count the number of blood vessels, osteoclasts and Howship lacunae present in the periodontal ligament (PDL) adjacent to the mesiobuccal roots of the maxillary right first molar. The slides were analyzed under light microscopy at

an original magnification of 200x. The histological criteria used to identify osteoclastic cells were the presence of eosinophilic and multinucleate cells (19).

The slides stained with picosirius were analyzed under polarized light microscopy at an original magnification of 100x to determine the structural changes in the collagen present in the new bone formation of trabecular matrix, adjacent to the traction side of the mesiobuccal root of the maxillary right first molar. This method allows an indirect assessment of the organization stage of the bone matrix based on the birefringence of the collagen fiber bundles. The mature collagen presented reddish tones while immature collagen was yellowish-green. The analysis was performed using Image-Pro Plus 4.5 image-analysis software (Media Cybernetics, Silver Spring, MD, USA), which calculated the percentage of mature and immature collagen. Measurement was performed in a randomly selected field of each section, and a mean of the percentage for each animal was obtained.

The Kruskal Wallis test was used to compare the intergroup variables at a significance level of 5%.

Results

Control Group

On the 3rd day, PDL presented with disorganized collagen fiber (CF) bundles in the compression area, with a predominance of oval fibroblasts (FB) and intense vascularization (Fig. 2A, Table 1). An increase in the number of osteoclasts (OC) located inside the Howship lacunae (HL) and contiguous to the bone surface was observed. In the traction area, PDL exhibited stretched, oblique and parallel CF bundles among them with a predominance of fusiform FB and moderate vascularization (Fig. 2C, Table 1). Under



Figure 1. Orthodontic appliance fixed in the oral cavity of the rat.

polarized light microscopy, there was a preponderance of immature collagen and the beginning of collagen matrix production (Table 2). After the initial stage of tooth movement, PDL thickness on both sides showed a trend towards normality on the 14th and 21st days. In the compression area, the CF bundles were disorganized with predominance of oval FB (Fig. 2E, I, M, Table 1) while in the traction area the CF bundles were stretched, oblique and parallel among them with predominance of fusiform FB dispersed in the matrix (Fig. 2G, K, O, Table 1). Gradual decrease occurred in the number of blood vessels (BV) from the 7th up to the 21st day with predominance in the number of OC on the 7th day, which was not identified with the same frequency as in the earlier periods (Table 1). In the analysis under polarized light, a greater organization and increase of mature CF with reddish coloration was observed on the 7th day. Evidence of higher levels of collagen matrix synthesis, with expression of more organized fiber was

found in the final stages of tooth movement (Table 2).

Experimental Group

From the 3rd to the 21st day, the compression and traction areas presented rare BV. In the compression area, the CF bundles were disorganized with predominance of oval FB, while in the traction area the bundles were stretched, oblique and parallel among them permeated by fusiform FB. The alveolar bone showed resorption units consisting of numerous OC, showing intense resorptive activity on the 3rd day (Fig. 2B, Table 1). Afterwards, the bone surface was irregular with the presence of HL containing OC on the 7th and 14th days (Fig. 2F, 2J, Table 1). On the 21st day occasional OC and HL were observed (Fig. 2N, Table 1). Under polarized light microscopy, the matrix was constituted of mature collagen with more evident organizational level than in CG (Table 2).

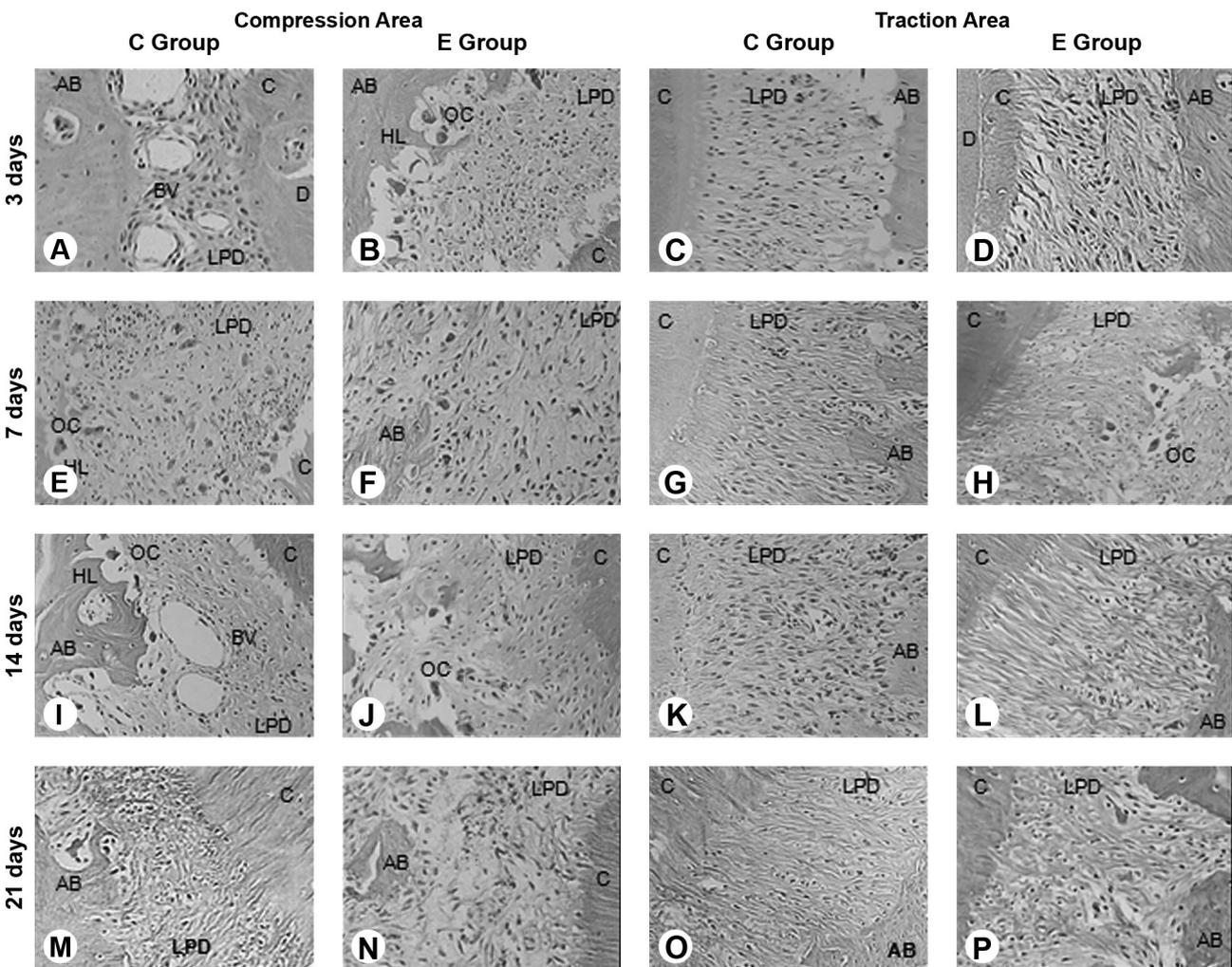


Figure 2. Compression (A, B, E, F, I, J, M, N) and traction area (C, D, G, H, K, L, O, P) of mesiobuccal roots of control group in 3rd (A, C), 7th (E, G), 14th (I, K), 21st day (M, O) and of experimental group in 3rd (B, D), 7th (F, H), 14th (J, L) and 21st day (N, P). Cementum (C), dentin (D), alveolar bone (AB), periodontal ligament (PDL), blood vessel (BV), osteoclast (OC) and Howship lacunae (LH). HE, 200x.

The morphological characteristics of tissues and cells found in group C correspond to a typical biological response to mechanical forces produced by the orthodontic tooth movement on PDL and alveolar bone, with reference to the studies of Heller and Nanda (18) and Macapanan et al. (20). The comparison of this group with group E shows that the administration of GH induced dynamic changes in PDL and alveolar bone in experimentally moved rat teeth.

It must be considered that the 30 cN force level selected for this study produced excellent force, because it caused effective induction of tooth movement without causing

any adverse effect in control group, such as the formation of hyaline areas (13). The time during which the appliance remained in the oral cavity was based on a previous study showing that 10 to 14 days are needed for a complete cycle of PDL bone remodeling in rats (21). Although GH is widely indicated and used, there are no studies reporting its effects during tooth movement.

In EG, there was predominance of OC on the 3rd day and decline in the subsequent periods with a significant difference on the 3rd and 14th days compared with CG (p<0.05). These results corroborate those of Tresguerres

et al. (3), who verified an increase in bone resorption after 1 week of local application of GH in periimplant tissue. Previous studies (11-13) reported that GH accentuated bone remodeling turnover, stimulating resorption and osteoblast activation, resulting in new bone formation.

In EG, a significant decrease was observed in the number of BV compared with CG on the 3rd and 7th days. There are few studies that directly verified whether GH modulates angiogenesis, but there is evidence that when there is GH deficiency or excess, the vascular function could be modified (22). The polarization method combined with computerized analysis is useful to assess the GH effect in orthodontic movements and allows the correlation of the structural organization of CF (23) with the area of primary bone osteogenesis. The polarization method has been used as a possible indicator of collagen aggregation (23), but there are no studies in the literature using the picosirius method in experiments related to orthodontic movement.

In CG, morphometric analysis on the traction side showed immature CF in the initial stages, with their progressive maturation in the final stages. However, in EG, only mature collagen was observed, and when compared with group C, in the periods of 3 and 7 days, significant difference was shown. However, *in vitro* studies indicate that GH can act directly on the osteoblasts and exert anabolic effects on bone formation (11,12).

This divergence could be explained by the fact that GH replacement may cause a two-phase effect having an initial predominance of bone resorption, and after

Table 1. Results for the number of blood vessels, osteoclasts and Howship lacunae at the four evaluation periods and intergroup comparisons (Kruskal Wallis test, p<0.05)

Variable	Day	Control group			Experimental group			p value
		Mean	Median	S.D.	Mean	Median	S.D.	
Osteoclasts	3	6.3	6.5	1.337	31.2	31.5	3.225	0.000*
	7	16.9	17	3.348	19	19	4.714	1.000
	14	3.3	3.5	1.059	14.8	14	2.348	0.036*
	21	1.4	1	1.265	5.3	6	2.359	1.000
Howship lacunae	3	6.5	6.5	1.958	43.1	43.5	10.027	0.000*
	7	17.8	17.5	2.573	24.8	23	7.068	1.000
	14	3.9	4	1.197	15.7	14.5	4.398	0.145
Blood vessels	21	1	1	0.943	5.2	5.5	2.616	1.000
	3	25.5	25.5	1.958	0.7	0.5	0.949	0.000*
	7	7.1	7	1.449	0.8	0	1.135	0.000*
	14	3.1	3	1.197	1	0	1.333	0.936
	21	3.1	3	1.101	2.1	2	1.853	1.000

S.D.: Standard deviation. *Statistically significant values if p<0.05.

Table 2. Results for the amount of mature and immature collagen at the four evaluation periods and intergroup comparisons (Kruskal Wallis test, p<0.05)

Variable	Day	Control group			Experimental group			p value
		Mean	Median	S.D.	Mean	Median	S.D.	
% mature collagen	3	10.5	11.0	0.898	100.0	100.0	0.000	0.000*
	7	38.3	37.5	1.359	100.0	100.0	0.000	0.021*
	14	100.0	100.0	0.000	100.0	100.0	0.000	1.000
	21	100.0	100.0	0.000	100.0	100.0	0.000	1.000
% immature collagen	3	89.5	89.0	0.898	0.0	0.0	0.000	0.000*
	7	61.7	62.5	1.359	0.0	0.0	0.000	0.021*
	14	0.0	0.0	0.000	0.0	0.0	0.000	1.000
	21	0.0	0.0	0.000	0.0	0.0	0.000	1.000

S.D.: Standard deviation. *Statistically significant values if p<0.05.

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12 to 24 months of treatment, stimulation of new bone formation with subsequent bone mass gain may occur (6, 24). Considering these changes in the bone metabolism, it was noted that a long time of observation is needed to obtain new bone formation.

These results suggest the hypothesis that GH could have the ability to stimulate resorption and, probably, delay new bone formation resulting from diminished vascularization. It is known that vascular proliferation is observed in the PDL of orthodontically moved teeth, favoring the regenerative processes characteristic of this region, and is an important mediator in the bone remodeling process (25).

It is suggested that individuals undergoing orthodontic treatment and who use GH, require longer intervals between the applications of light orthodontic forces, since the new bone formation process is delayed, and because they present more intense bone resorption, particularly in the initial stages of administration of the drug. Another recommendation would be to begin orthodontic treatment after the initial stage of GH administration, since it stimulates bone formation only after 12 to 24 months. Radiographic control must be frequently made to assess the status of bone resorption.

It was concluded that GH accelerated and intensified bone resorption and delayed immature collagen formation after experimental tooth movement in rats.

Resumo

O objetivo deste estudo foi avaliar histologicamente o efeito do hormônio de crescimento (HC) no ligamento periodontal e osso alveolar, durante a movimentação dentária induzida em ratos. Oitenta ratos Wistar, machos, divididos nos grupos controle e experimental, foram observados nos dias 3, 7, 14 e 21. Foi aplicada força ortodôntica (30 cN) sobre o primeiro molar superior por meio de um dispositivo ortodôntico. No grupo experimental foi administrada 0,1 UI/kg/dia de HC e, no grupo controle, 0,5 mL/kg/dia de solução salina. As amostras foram processadas e avaliadas por microscopia de luz e luz polarizada. O teste de Kruskal Wallis foi utilizado para a comparação das variáveis intergrupos. Verificou-se que o grupo experimental apresentou maior quantidade de osteoclastos nos 3º e 7º dias e de lacunas de Howship no 3º dia, menor quantidade de vasos sanguíneos e maior quantidade de colágeno maduro nos 3º e 7º dias do que no grupo controle ($p < 0,05$). Concluiu-se que o HC acelerou e intensificou a reabsorção óssea e produziu atraso na formação de colágeno imaturo, durante o movimento ortodôntico induzido.

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