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INFLUENCE OF MYCOPHENOLATE MOFETIL AND SIROLIMUS IMMUNOSUPPRESSIVE DRUGS ON ORTHODONTIC DOGS' TOOTH MOVEMENTS

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ABSTRACT:

Introduction: *The purpose of this study was aimed to evaluate the effects of administering two immunosuppressive drugs, mycophenolate mofetil and sirolimus on orthodontic tooth movement in dogs.*

Methods: *Fifteen adult healthy dogs were equally divided into three groups, group1, the control did not receive any drugs while dogs in groups 2 and 3 received mycophenolate mofetil and sirolimus, respectively. A 100 gm force was applied to the left and right sides of the maxillae in all groups using nickel titanium coil spring attached to the lateral incisors and canines for 42 days. Tooth movement was assessed by measuring differences in the distance between the lateral incisors and canines on days 0 and 42. At day 42, the dogs were euthanized and the maxillae were dissected, teeth and the surrounding alveolar bone were processed for histological examination.*

Results: *Histological results showed newly formed bone at the tension side of the control group while the pattern of bone resorption seen at the pressure side indicated the presence of front and direct bone resorption. Less bone deposition and resorption were observed at the*

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tension and compression sides in mycophenolate mofetil treated dogs while in the tension side of sirolimus treated dogs, little or no bone apposition was present and similarly, little or no bone desorption was observed at compression sides. Moreover, there was a statistically significant difference between the tooth movement among the three groups at day 42 ($p<0.001$). Within each group, there was a significant difference between tooth movement at days 0 and 42 in groups 1 and 2 ($p=0.05$) but group 3 showed no significant difference ($p>0.05$)

Conclusions: *Both mycophenolate mofetil and SLR significantly altered orthodontic tooth movement; however mycophenolate mofetil seems to be less disruptive than sirolimus to the pattern of orthodontic tooth movement.*

INTRODUCTION

Orthodontic tooth movement is based on application of a force to a tooth through orthodontic devices. This force will result in remodeling changes in both dental and paradental tissues leading to tooth movement.[1] Orthodontic forces change the blood flow and the electrochemical balance of the periodontal ligament.[2] Consequently, initiation of cellular and biochemical reactions causing bone resorption at the compressed side and deposition in the tension side of periodontal ligaments.[3,4]

The early phase of orthodontic tooth movements shows an acute inflammatory response, including vasodilatation of the periodontal vessels and migration of leucocytes out of capillaries. These cells produce cytokines that interact with other systemic and local molecules promoting secretion of prostaglandin and growth factors.[5] Different studies proved the direct effect of prostaglandin on bone resorption.[6,7] It increases the number of osteoclasts, enhances their capacity to form ruffled borders and stimulates bone resorption.[8]

It is well established that tooth movements are influenced by many factors such as force magnitude, force duration, force decay, age, general health of the patient, and pharmacological agents.[9] Osteoclasts inhibition by indomethacin through its inhibition of cyclo-oxygenase which is

required for prostaglandin synthesis and hence inhibit tooth displacement.[10,11] Vitamin D_3, corticosteroid hormones, parathyroid hormone, thryroxine and eicosanoids are considered as a tooth movement's stimulants. On the other side, non-steroidal anti-inflammatory drugs, estrogens and bisphosphonates decrease tooth movements.[12-14]

Immunosuppressive drugs are prescribed to those patients with organ transplant to avoid graft rejection. The number of kidney transplant cases increased at Mansoura University Urology and Nephrology Center reaching more than 100 cases per year. The children represent about 60% of the total transplant cases.[15] Among the newly prescribed drugs is the mycophenolate mofetil and sirolimus. Mycophenolate mofetil is an ester and hydrolyzed to mycophenolate acid (MPA), the active form of the drug. mycophenolate mofetil inhibits the inosine monophosphate dehydrogenase that is an important enzyme in purine synthesis.[16,17]

Meanwhile, sirolimus is a lipophillic microcyclic lactone. sirolimus binds protein 12 (FKBP-12) and forms an immunophillin complex that acts as a catalyst. This complex targets the serine-threonine kinase of the phosphatidylinositol-3-pathway called mTor. MTor has been found as the principal controller of cell growth and proliferation.[18-21] Based on the above, the present study aimed to assess the effects of mycophenolate mofetil and sirolimus on orthodontic tooth movement in mongrel dogs.

MATERIAL AND METHODS

Fifteen adult healthy male, Mongrel dogs, between 8 and 10 months old were used .The dogs were individually caged in facility with controlled light and temperature and were fed soft food. The dogs were equally divided into three groups (n=5). Group 1, was control where the dogs did not receive any medication while dogs of groups 2 and 3 received mycophenolate mofetil (Cellcept, Roche laboratory, Nutley, NJ, USA), 10 mg/kg/day[22] and sirolimus (Rapamune, Wyeth, 5 Giralda Farms, Madison, NJ, USA), 1 mg/kg/day[16] respectively.

The experimental protocol was conducted according to the ethical guidelines for animal care of our university. Each animal was sedated with an intramuscular injection of 1 mg/Kg xylazine. General anesthesia

was induced with an intramuscular injection of 6-mg/Kg thiopentone. Before the beginning of all experimental procedures the trachea was intubated and general anesthesia was maintained using halothane (1.5–2.5%) in oxygen, delivered through a semi-closed breathing circuit.[23]

A NiTi coil spring (GAC international, Inc, New York, USA) was placed in the right and in the left sides of dog's maxilla between lateral incisor and canine and was activated to produce 100 gm measured with (Correx, Koeniz, Swiss) to pull the incisor distally. The coil spring was ligated with a 0.25 mm ligature wire (Dentaurum, Pforzheim, Germany) through a notch prepared in the neck portion of both canine and lateral incisor teeth. The notch was prepared using a round rose head bur (US No.332 Komet, Germany) and a micro motor (W&H, Austria) to avoid displacement of the ligature wire.[24] Any sharp edges of the wire were cut and wire ends were bent to avoid soft tissue injury (Fig1). The lower canines were sectioned horizontally along the cervical one third and endodontically treated to be contact free with the upper lateral incisor and canine.[25]

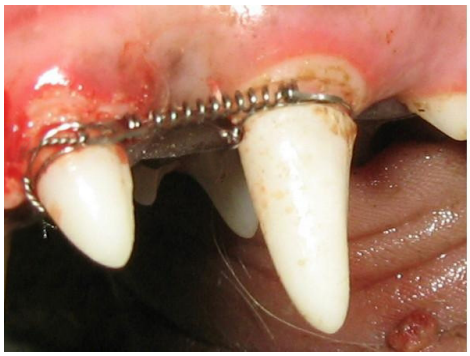

Fig 1. Orthodontic appliance in place.

The distance between the most mesial point of the canines and the most distal point of the lateral incisors at the gingival level was measured in all groups. Measurements were taken bilaterally i.e. on both right and left sides of the dogs' maxilla. A digitronic caliper (Wilson and Wolpert, Utrcht, Netherlands) was used to measure the distance on days 0 and 42, while the animals were anaesthetized. All measurements were made twice within a period of few minutes. Tooth movement was calculated by

subtracting distance between the teeth on day 42 from the distance between the teeth on day 0.[26]

At the end of the experiment, after 42 days, the animals were anaesthetized with sodium pentobarbital and intra-cardially perfused with 4% paraformaldehyde in 0.2 M phosphate buffer containing 0.2% picric acid. With an oscillating saw, maxillae were cut and sectioned along the mid palatal plane to be divided into left and right hemi-maxillae and the selected teeth with their surrounding bone were excised. The specimens were decalcified in 10% EDTA (Tritriplex III, Merckx-Belgolabo, Belgium) in 0.1 M phosphate buffer for 2 weeks and embedded in paraffin wax. The right and left upper lateral incisors were cut mesiodistally into 5-µm-thick sections. The sections were stained with hematoxylin and eosin, for histological examination. Using an optical light microscope (Zeiss, Goettingen, Germany), specimens were examined as coded slides by one observer to avoid potential viewer bias.

Data Analysis:

Descriptive statistics of the distance (mm) between the lateral incisors and canine both sides of each dog's maxilla at day 0 and day 42, including the mean, standard deviation were calculated using the SPSS (Statistical Package for the Social Sciences, Chicago, Ill) program. Kruskal –Wallis and Mann-Whitney tests were performed to determine whether a significant difference existed between the various groups. Wilconson Signed ranks test was done to evaluate significant difference within each group between day 0 and day 42.

RESULTS

Histological Results:

The hematoxylin & eosin stained sections of Mongrel dogs' upper lateral incisors alveolar crest region at the tension side (Fig 2A) of the control group showed the width of the PDL increased, both fibroblasts and osteoblasts were found to be more evident. The blood vessels with a round-oval or tubular shape and the newly formed bone can be clearly seen (arrows) while the PDL in the compression side Fig (2B) of that

group was narrowed with an increase in the vascular activity and leakage of blood constituents into the extra vascular space, resorption lacunae could be observed and osteoclasts were found to be more evident (arrows) suggesting front and direct bone resorption. In the tension side (Fig 2C) of mycophenolate mofetil-treated group, the PDL was less organized than in the control group with a low level of vascularity, and decreased fibroblast, osteoblast cellularity as well as a lesser amount of newly formed bone indicating less deposition (arrows). However, in the compression side (Fig 2D) of that group, lesser vascularity and decreased osteoclasts number in limited resorption lacunae (arrows). The PDL in the tension side (Fig 2E) of sirolimus-treated group was more disorganized with fewer fibroblast and osteoblast cells as well as hyalinized areas of glassy appearance (arrows) and little or no bone deposition. In the compression side (Fig 2F) of that group, the PDL was compacted and the osteoclasts were not prominently seen little or no bone resorption (arrows). No root resorption could be seen in all groups.

Statistical Results:

Means and standard deviations of the distance between lateral incisor and canine (mm) at day 0 and day 42 are presented in (Table 1). The mean difference was 2.65 ±0.624mm in group 1, 1.640± 0.37mm in group 2 and 0.6 ±0.09mm in group 3. Kruskal Wallis test found no significant difference between the three groups at day 0 ($p>0.05$), while there was a significant difference between the three groups at day 42 ($p<0.001$) (Table 2).These results were further analyzed by Mann-Whitney test that showed a significant difference between group1-2 ($p=0.001$), group 1-3 ($p=0.002$), and group 2-3 ($p<0.001$) at day 42. Also, there was no significant difference between the above groups at day 0 ($p>0.05$) (Table 3). Meanwhile, Wilcoxon Signed Ranks test showed that, within each group there was a significant difference between day 0 and day 42 in both groups 1 and 2 ($p=0.005$).On the other hand, there was no significant difference between day 0 and day 42 in group 3 ($p>0.05$) (Table 4).

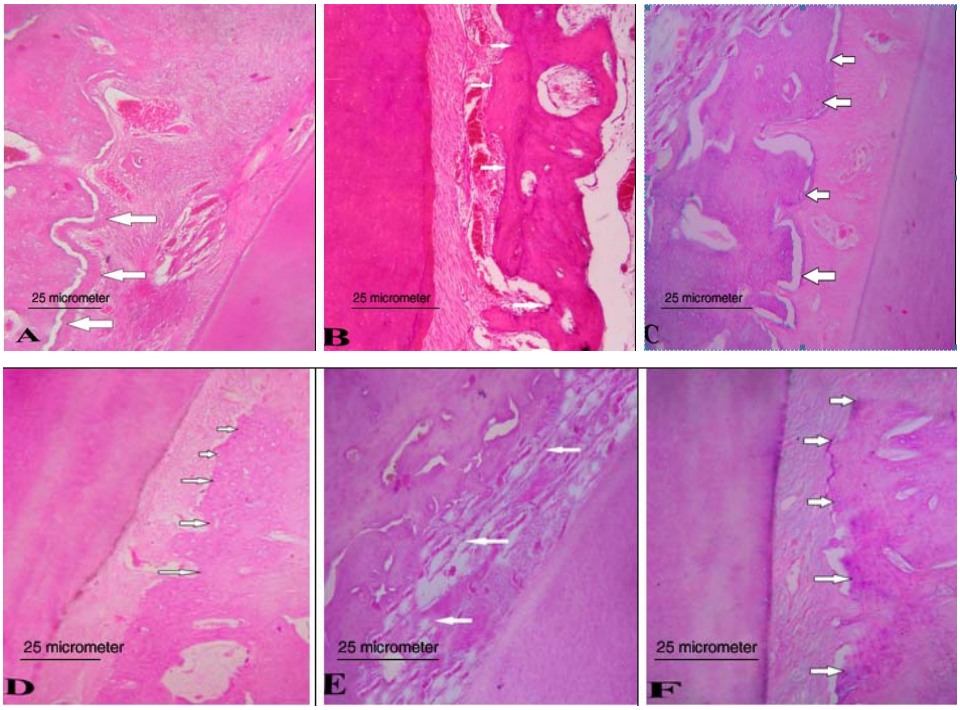

Fig 2: Light microscopic observations of Mongrel dog upper lateral incisor alveolar crest region (hematoxylin & eosin stain 200x) showing tension side of the mechanically activated control group (A), compression side of that group (B), tension side of mycophenolate mofetil-treated group (C), compression side of that group (D), tension side of sirolimus treated group (E) and the compression side of that group (F). Arrows indicate the areas of resorption and deposition of bone for each group.

Table 1: Means and standard deviations of the distance (mm) between maxillary lateral incisor and canine in the three tested groups at day 0 and day 42 and their difference.

Groups	Mean			Standard Deviation		
	Day 0	Day 42	Difference	Day 0	Day 42	Difference
Group 1	12.750	10.1	2.65	0.679	0.624	0.624
Group 2	13.01	11.370	1.64	0.626	0.702	0.372
Group 3	12.74	12.68	0.6	0.75	0.750	0.097

Table 2: Results of Kruskal-Wallis test between day 0 and day 42 among the three tested groups.

Periods	Chi-Square	df	Significance
Day 0	1.618	2	0.445
Day 42	21.522	2	0.000
Difference	24.160	2	0.000

Table 3: Results of Mann-Whitney test between day 0 and day 42 among the three groups.

Groups	Mann-Whitney U			Significance		
	Day 0	Day 42	Difference	Day 0	Day 42	Difference
Group 1-2	34	8	8	0.247	0.001	0.001
Group 2-3	37	10	0.00	0.353	0.002	0.000
Group 1-3	49	0.00	0.00	0.971	0.000	0.000

Table 4: Results of Wilcoxon Signed Ranks test between day 0 and day 42 within each group.

Groups	Mean Rank	Sum of Ranks	Significance
Group 1	5.5	55	0.005
Group 2	5.5	55	0.005
Group 3	2.5	10	0.59

DISCUSSION

Orthodontic tooth movement comprises of four phases. In phase 1, which lasts approximately 24 hours to 2 days, the tooth is displaced within its alveolar bone socket. Phase 2, arrest phase, little or almost no tooth movement is present and this attributed to hyalinization. Phase 2 can last up to 20-30 days. At the end of this phase, necrotic tissues are removed , tooth movements accelerates in the third phase and continues in a linear pattern in the forth. [27] In this study, the duration of 42 days was chosen to show changes that may occur during the active phase of tooth movements.

A 100 gram of force was selected in this study, as the previous study found no significant difference in the rate of tooth movements between 50, 100, or 200 gm.[28]Lighter forces produce favorable tooth displacement, resulting in minimal discomfort and pain. Differences between PDL and alveolar bone of dogs and human beings are quite small and hence considered as good model for comparison with human being.[2]

Statistical analysis of the data in this study showed a significant difference in the tooth movement between group 1-2 ($p=0.001$), group 1-3 ($p=0.002$), and group 2-3 ($p<0.001$) at day 42. Also, there was no significant difference between the above groups at day 0 ($p>0.05$). Meanwhile, within each group there was a significant difference between tooth movement at day 0 and day 42 in both groups 1 and 2 ($p=0.05$). While there was no significant difference between the movement at day 0 and day 42 in group 3 ($p>0.05$).

The histological findings of the present study supported and explained the aforementioned statistical results. Bone deposition and bone resorption were seen at tension and compression sides respectively in the mechanically control group(group 1). This is in accordance with Sandstedt as cited by Meikle[30] and Schwarz[31] who stated, tooth movements occurs in the periodontal space through producing a "pressure side" and a "tension side." Differentiation of osteoclasts occurs, and they resorb bone of the socket wall on the pressure side. At the same time, remodeling of collagen fiber in the PDL occurs to accommodate the new tooth position. On the tension side, remodeling of collagen fibers bundles also takes place but in association with alveolar bone deposition.

These results can be assured upon the fact that, with pressure, vascular constriction, cellular and fiber production decrease and consequently, PDL becomes disorganized. On the tension side, widening of PDL space occurs with increased cellularity and collagenous fiber synthesis as well as subsequent alveolar bone deposition is observed. [5]

Compared to the control group, decreased vascularity and osteoblastic content resulted in less deposition of new alveolar bone and decreased osteoclasts number indicating less alveolar bone resorption in the tension and compression sides of mycophenolate mofetil group

respectively. Hence, less tooth movement was recorded in group 2 (mean 1.64±0.37mm) compared to group 1 (mean 2.65±0.62mm). However,in contrast to the destructive effects of mycophenolate mofetil on the PDL and surrounding alveolar bone were substantially less in comparison with the sirolimus treated group which showed the least amount of tooth movement (mean 0.6±0.09mm).

These pervious findings can be explained on the basis that mycophenolate mofetil has a selective antiproliferative and immunosuppressive activity affecting only lymphocytes as reported by Eugui et al[35], who observed that a lymphocyte-selective antiproliferative and immunosuppressive activity of mycophenolic acid (MPA) and its morpholinoethyl ester in mice and rodents.[32,33] The above explanation was further elucidated by Ransom[36]; Voisard et al[37]; Ji et al[38], who reported that the lymphocytes are more dependants on de novo synthesis of purines than other cells which reclaim purines by savage pathway. MPA suppress dendritic cell maturation and can induce human monocyte-macrophage cell line differentiation, decreasing the expression of interlukin IL-1 and enhancing expression of IL-1 receptor antagonist.[34-36]

On the other hand, The PDL in the tension side of sirolimus-treated group was more disorganized with hyalinized areas and reduced numbers of fibroblast and osteoblast cells which account for the observed little or no alveolar bone deposition. While on the compression side, the lack of evident bone resorption and almost absence of osteoclasts can be attributed to the sirolimus antiproliferative effects that include both the immune and nonimmune cells. This is consistent with the finding of Francavilla et al.[37], Gregory et al.[38] and Poon et al.[39] who concluded that the characteristic feature of sirolimus is its ability to inhibit growth factor signaling for both immune and nonimmune cells which include fibroblasts and endothelial cells, hepatocytes, and smooth muscle cells.[37-39]

Additionally, these results can be attributed to the mechanism of action of sirolimus and was clarified with Chen et al., Marx et al., and Sabatini et al. who consider sirolimus is a lipophillic that passes through cell membranes easily, and the segment of the macrolactam ring identical to tacrolimus binds to cytosolic FK506-binding proteins (FKBP). The consequent mechanisms of action for tacrolimus-FKBP12 and sirolimus -FKBP

complexes differ in several ways. Unlike tacrolimus, sirolimus does not inhibit calcineurin phosphatase, but its molecular targets include RAFT1/FRAP proteins in mammalian cells, associated with cell cycle progression through G1; however, the exact mechanism of inhibition of cell cycle progression through these proteins is still unknown.[40,41]

CONCLUSIONS

According to the present histological and statistical results, it is concluded that administration of mycophenolate mofetil and sirolimus immunosuppressive drugs significantly affect orthodontic tooth movements. It is further concluded that, the impairments of mycophenolate mofetil were less compared to the sirolimus and hence, mycophenolate mofetil drug is considered a better choice than sirolimus for orthodontic patients who are simultaneously undergoing immunosuppressive drug therapy. A further study is recommended to evaluate the long term effect of mycophenolate mofetil and sirolimus immunosuppressive drugs on the pattern of orthodontic tooth movements.

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