INHIBITORY EFFECT OF BISPHOSPHONATE [PAMIDRONATE] ON ORTHODONTIC TOOTH MOVEMENT IN NEWZEALAND ALBINO RABBITS

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Abstract

In orthodontics attaining absolute anchorage is one of the greater tasks for successful outcome of the treatment. The purpose of this study was to investigate the inhibitory effect of locally administered bisphosphonate – pamidronate on orthodontic tooth movement (OTM) in Newzealand albino male rabbits which could support the 'Pharmacological Anchorage System' i.e. preventing unwanted tooth movement to accomplish orthodontic anchorage by local administration of certain drugs.

The present experimental study was carried out for '21' days on '20' rabbits [10- Control and 10- Experimental] with 3.75 to 4 kg wt. On the 1st day the animals received orthodontic appliance between 1st molar and incisors of the mandible. On day 1, 7 & 14, control animals received normal saline and experimental animals received Bp- pamidronate 1.5mg/0.5ml locally. After 21days the animals were sacrificed and the mandibles were dissected. The 1st molars with intact mesial aspect of alveolar bone were sectioned and processed for histological study and performed osteoclastic count under light microscope. Student 't' test was used to compare the two groups and found significant ($P<0.001$) difference between two groups.

The present study concluded that locally administered Bp (pamidronate) had shown decreased osteoclastic count and reduced tooth movement.

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Introduction

Orthodontic forces are generated by orthodontic appliances which are applied on teeth for the purpose of effective tooth movement in a desired mode. Orthodontic tooth movement (OTM) is a response to mechanical stimuli which is a resultant of sequential reactions occurring in the periodontal tissue and alveolar bone due to the release of various chemical substances from the dental tissues and neighboring structures.[1-3]

In orthodontics, generally during OTM two units are planned for the effective tooth movement; one as anchoring unit another as moving unit. In the evolution of different orthodontic appliance designs 'anchorage preparation' has retained its own importance in the treatment plan for effective treatment

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outcome. Various mechanical approaches like skeletal anchorage by zygomatic ligatures[4], prosthetic implants[5], palatal implants[6] etc. have been implicated for anchorage enhancement in orthodontics; these have drawbacks like increased chair side time, invasive method etc. Recently, mini/micro implants have become popularized and gained prime importance in achieving absolute anchorage[7,8], but still these implants also have certain drawbacks like poor patient cooperation , damaging anatomical structures , inflammatory changes ,questionable success rate etc. To overcome drawbacks of mini implants and mechanical devices, recent researchers are inclining towards different approach to promote anchorage preparation with the help of biological inhibitors of osteoclastic bone resorption which is termed as 'pharmacological anchorage system[9].

Drugs like NSAID's- indomethacin[10], Acetyl salicylic acid[11,12], ibuprofen[12] and diclofenac sodium[13], Bps[9] etc., have shown inhibitory effect on OTM, which are categorized as inhibitors of OTM; contrarily another group of drugs like - Corticosteroids in dose dependant manner[14], local application of Vit-D_3[15], PTH hormones[16], Prostaglandins[17], cytokines[18] etc., have shown enhancive effect on OTM by local administration, which are categorized as promoters of OTM. Among all these pharmacological agents most commonly prescribed medicines during orthodontic treatment course are NSAIDs, these have negative effect on OTM, where as acetaminophen exhibit neutral effect on OTM which may be a choice of drug for orthodontic patients for relieving pain[19,20].

In modern day, orthodontic practice is not only limited to healthy and younger individuals, but also to patients under medication therapy for various disorders, who are also opting for orthodontic treatment. Bps are anti bone resorptive drugs indicated in various skeletal disorders like prevention and treatment of osteoporosis, Paget's disease, multiple myeloma, hyper parathyrodism, osteogenesis imperfect etc. Bps shares a common chemical structure, in which two PO3 (phosphate) groups are bonded to carbon atoms. Bps are analogs of inorganic pyrophosphate. There are Non-Nitrogenous Bps and Nitrogenous Bps. Non-nitrogenous Bps includes etidronate, clodronate and tiludronate category. They are incorporated into non-hydrolysable analogues of ATP, interfering with ATP-dependent pathways and resulting in osteoclast apoptosis[21]. Nitrogenous Bps includes pamidronate, alendronate, zolendronate, residronate and ibandronate. These are more potent than non nitrogen-containing Bps. They Inhibit osteoclast farnesyl pyrophosphate synthase (FPPS) and prevent protein prenylation required for the formation of GTPases required by the osteoclast. The end result is osteoclast apoptosis[22]. The ultimate goal of either group of Bps are antiresorptive activity by osteoclastic apoptosis.

Orthodontists should pay special attention for patients on Bp therapy undergoing orthodontic treatment because the antiosteoclastic activity of these drugs might interfere with OTM and shows negative impact on treatment outcomes like difficulty in retraction of teeth followed by extraction space closure, retarded tooth movement, increase relapse tendency, increase treatment duration[23,24]. The inhibitory (unfavorable) effect of Bps can be utilized for favorable purpose to promote the anchorage and prevention of relapse in experimental animals[25,26].

Pamidronate belongs to nitrogenous group of Bp and is utilized in this study which could interfere with bone remodeling and exert negative influence on OTM by depleting osteoclast count, thereby decreasing molar tooth movement. The purpose of this study was to examine the effect of local administration of Bp (pamidronate) on OTM in Newzealand albino rabbits.

Methods

The present animal experimental study protocol has been approved by the institutional animal ethical committee (IAEC) - Approval no: 160/99/CPSEA, Prop.no:854 of Annamalai University, Tamilnadu, India. Total 20 Newzealand albino rabbits used in this study were supplied by veterinarian staff incharge of Annamalai University, which were bred in the reproductive lobby of the animal house. Total '20'animals were segregated into '2' groups. One group of '10'animals served as Control – received normal saline locally and another group of '10'animals served as Experimental- received Bp (pamidronate) locally.

Materials Used

The materials used for this study are Pamidronate I.V-2.5ml (Biodronate 30 Pamidronate Disodium for I.V.infusion by United Biotech Pvt Ltd , India), LED Cure Light, Ketamine injection 35mg/kg, Light cure Transbond XT-3m Unitek, Saline Solution-0.9%-Baxter ,Primer Transbond XT-3m Unitek, Disposable syringe-2.5ml, Flowable gel based Etchant-Mission Dental USA, NiTi Coil Spring-100gms force, Stainless Steel Ligature wire-0.009" and Self Designed Custom made Mouth prop.

Animals Maintainance

In this experimental study '20'Newzealand albino rabbits were used. All animals were bred and supplied by animal house of Annamalai University, India. All male rabbits with average weight of 3.5 to 4 K.G wt, 16 – 18 weeks old were chosen for this study.

Throughout this experimental study animals were examined daily by veterinarian staff and us. Before commencing of this experiment, one week earlier all animals were kept in separate metallic cages for adaptation of animal house environment.

The animals were maintained at room temperature between 20 and 25^{0}C with constant humidity fed with standard ration and water. These animals have been selected for the experiment due to its easy nature of maintenance, feeding and above all installation of the appliance was least cumbersome when compared to other small rodents.

Drugs Used

The animals in each group were anaesthetized on the first day by an intramuscular injection of Ketamine (50mg/kg body wt) and Intramuscular injection of diazepam (5mg/kg body wt) for appliance placement. Bisphosphonate- Pamidronte 1.5mg/0.5ml injection was used for experimental group and 0.5ml saline for control animals. Each time the animals were anaesthetized before local administration of pamidronate .

Experimental Procedure

Before administration of saline (Control group) and the drug (Experimental group), the self designed custom mouth prop fabricated with 19 gauge SS wire was inserted so that placement of the appliance would be easier. Small grooves were made around the incisors and molars in the lower jaw with the help of a round bur at 1.5-2mm above the gingival margin and saline based coolant was used with suction. A stainless steel ligature wire of 0.009" dimension was wound circumferentially around the molars and incisors; now a 100gms force NiTi Coil spring was tied between the incisors and lower right first molar region and both ends of ligature wires were twisted with artery forceps.

Before ligation the force was measured by using an electronic force gauge which was attached to the end of the ligature wire and fitted in grooves, now the appliance was tied between both ligature wires.

Etching was done circumferentially around the incisors and 1st molar grooves and light cured with primer, above this composite applied and light cured. The experimental drug Pamidronte injection of 1.5mg/0.5ml was administered on the 1st, 7th and 14th day of the experiment and 0.5ml saline was given to control animals (Figure - I).

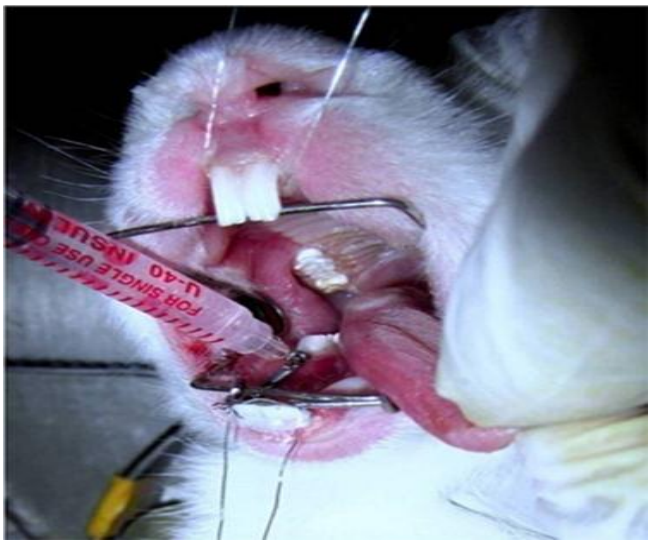


Figure I: Rabbit with appliance installed and receiving pamidronate local injection.

On 21st day animals were anesthetized same as above and euthanized with a 2ml intracardiac injection of potassium chloride[27]. All animals' mandibles were dissected along with appliance.

Histological Examination

Appliances were removed from dissected mandible, bony sections were separated from mesial to the 1st molar to distal to the 2nd molar with bone intact and stored in 10% formalin and immediately transported to oral and maxillofacial pathological laboratory, Faculty of Dentistry, Annamalai University. All samples were decalcified with 9% formic acid for an approximate period of one month. The progression of decalcification was observed with micro x-ray.

After decalcification the specimens including mandibular 1st molar along with alveolar bone (mesial aspect = compression side) were hemi sectioned at coronal, middle third and apical third at root level. Each section was again serially sectioned at 4 to 6 µm in the coronal plane. The sections were mounted on glass microscope slides and stained with hematoxylin and eosin. All histological examinations were focused on the osteoclastic count of alveolar bone in the mesial aspect of mandibular molar root at two levels i.e., coronal and middle third. The values obtained from these two fields (coronal and middle third) were averaged (Table-I).

ANIMALS	CONTROL	EXPERIMENTAL
1	13.8	5.6
2	13.0	5.9
3	14.2	4.9
4	13.2	6.5
5	13.8	6.4
6	13.4	6.1
7	11.5	5.1
8	13.9	5.9
9	13.0	6.1
10	13.5	5.9

Table-I: Control and Experimental Mean values of osteoclasts in coronal & middle 3rd

Field count was calculated and osteoclast numbers were expressed as per square millimeter. The multinuclear osteoclasts on the stained sections were seen with ruffled border which were counted by two oral pathologists twice at different times using light microscope. The method error was calculated by using Dahlberg's equation[15]. Error = 'I $Id^2/2n$ (d, difference between two measurements; n, number of samples). The experimental group animals have shown decreased osteoclasts when compared to non drug induced control group. [Figure : II A,B]

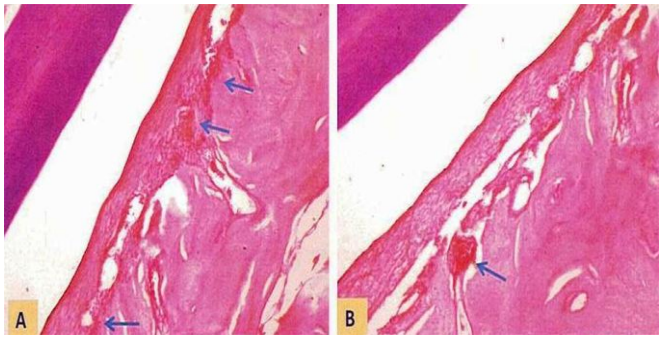


Figure II: A – Control Group – Mesial aspect of molar showing multiple osteoclasts (arrow) in the alveolar bone [Magnification x100, Hematoxylin and Eosin stain]

B – Experimental Group – Mesial aspect of molar showing decreased osteoclasts (arrow) in the alveolar bone [Magnification x100, Hematoxylin and Eosin stain]

Result

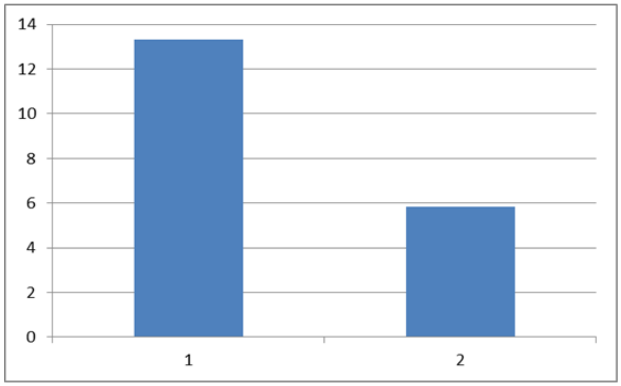
Statistical Analysis: The result of current study was reported as mean and standard deviation. The student (t) test was done between two means, obtained from two groups (Table-II, graph-I). If the p value is less than 0.05 ($p<0.05$), it is considered as significant difference between two groups.

The overall average values of osteoclastic count in the mesial aspect of 1st molar in control (13.33±0.756) and Experimental group (5.840±0.515) were statistically evaluated and found significant difference ($p < 0.001$), which was specified that depleted osteoclastic count was encountered in experimental samples than controls (Table-II, graph-I).

OSTEOCLAST COUNT	N	Mean	Std. Deviation	t- value	p- Value
Control	10	13.33	0.756	25.9030	0.001 (Significant)
Experimental	10	5.840	0.515		

Table-II: Student 't' test statistic analysis for mean values of osteoclastic count.

Inference: calculated P-value is significant at 0.001 level (P-Value <0.001). Therefore it is concluded that there is a significant difference between control and Experimental groups



Graph- I: Y axis – Osteoclastic count – Mean values in the mesial aspect of molars. X axis – 1:Control, 2: Experimental
Inference **-** the mean of control group (13.33) is higher than experimental (5.840) group.

The experimental group i.e. locally administered pamidronate animals have shown significant decreased osteoclastic count and decreased molar tooth movement when compared to non drug induced control group.

Discussion

The type and magnitude of force[28,29] and treatment duration[30] are main interplay factors to promote sound tooth movement and prevent unwanted tissue reactions such as sterile necrosis or root resorption. OTM is a response for an applied force on a tooth which is a resultant of sequential cellular and molecular responses taking place in the periodontal structures. Anchorage preparation is a greater task in orthodontic treatment plan in order to elicit OTM in a desired manner. In anchorage perspective various mechanical devices[4-6] and micro implants[7,8] have been introduced in orthodontics for effective tooth movement, but all these have their own drawbacks. In recent years investigators are developing interest towards "Pharmacological Anchorage System"[9] i.e. trying to accomplish anchorage by local administration of suppressive agents[9-13].

Among these suppressive agents Bps may be considered as superior drugs in controlling tooth movement and enhancing anchorage preparation because these medicines reduce bone resorption by selective adsorption on to the mineral surfaces of bone and interferes with bone resorbing osteoclasts by promoting apoptosis. The antiresorptive activity of Bp may be helpful in preventing tooth movement which is a favorable outcome in anchorage point of view in orthodontics which is sensible in designing this study. In this experiment bone resorption might have taken place in the mesial aspect of molars in response to the applied force on molar tooth and the locally used drug Bp (pamidronate) in the experimental animals has shown a considerable inhibitory effect on molar tooth movement which could be due to expression of anti resorptive effect of Bp in the local area and found depleted osteoclasts in drug induced animals.

There are some animal experimental studies that have been reported in the literature which were similar to the existing study; they are Igarashi et al[13], Kim et al[31], LinLiu et al[26], Seifi et al[32], Jeremy et al[33], and Yugi Fujimura et al[34]. In this study Newzealand albino rabbits were used because of ease of handling and comfortable placement of intraoral appliance compared to rats and mices.

Igarshi et al[13], studied the effect of topical administration of risedronate in rats and suggested that the topical administration of Bp (risedronate) could be helpful in the prevention of root resorption of teeth in the course of orthodontic treatment. Similarly in the current study also the local administration of Bp (pamidronate) in experimental animals might have prevented the root and alveolar bone resorption, thereby limited molar mesial drift was encountered.

Kim et al[31], stated that a single systemic administration of Bp (pamidronate) has decreased the extent of initial relapse in experimentally moved rat molars due to impairment of osteoclastic functions via apoptosis of osteoclasts.

LinLiu et al[26], gave local injection of Bp (Clodronate) in the bucco- mucoperiosteum region of left molars in Wister rats subjected to bilateral palatal expansion. They also stated that there was a greater amount of osteoclastic depletion in the local injection site on the left side. In the present study also histological examination has revealed reduction in the osteoclastic count in the alveolar bone of compression side of mandibular molar and reduced tooth movement.

Seifi et al[32], elucidated reduction of tooth movement and root resorption in the experimental study done in Wister rats following intraperitonial administration of Bp (pamidronate). The decreased amount of root resorption was evaluated by counting root resorptive lacunae under light microscope. Similarly in the existing

study the impeded molar tooth movement was observed on the dissected mandibles of drug administered rabbits and also decreased number of osteoclastic cells were found.

Jeremy et al[33], in their experimental study have demonstrated the inhibitory effect on mesial movement of molars in alendronate administered rats by 75% at 2 weeks and 58% at 4 weeks. The similar inhibitory effect was encountered on molar tooth movement in experimental animals of present study.

Yugi Fujimura et al[34], have studied the effect of local administration of Bp in mice and demonstrated the decreased osteoclastic activity which led to impediment of molar tooth movement. The result of the current study also demonstrated the significant declined osteoclast count in the experimental group of animals compared to control group.

CONCLUSION

Attaining simple and effective anchorage method is not an easy assignment in orthodontics. Local administration of Bp (pamidronate) following experimental induced molar tooth movement in albino rabbits has reduced alveolar bone resorption and the number of osteoclasts which led to decreased tooth movement.

To date various experimental studies have been performed on animal models to evaluate the effect of Bps on tooth movement and found inhibitory outcome, which may be applicable in near future to build up simple and effective “pharmacological anchorage system” to overcome the drawbacks of mini implants and mechanical anchorage devices. To apply this anchorage method in humans further numerous cellular and molecular level studies are essential

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