Histochemical Analysis of Acid-phosphatase Activity Incident to Orthodontic Tooth Movement in Albino Rats - An Experimental Study

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Article ID: WMC001653
Article Type: Research articles
Article URL: http://www.webmedcentral.com/article_view/1653
Subject Categories: ORTHODONTICS
Keywords: Acid Phosphatase Activity, Histochemical Analysis, Orthodontic Tooth Movement, Histological Analysis, Animal Study, Dental Tooth Movement

How to cite the article: Bhosale V, Hazarey P V, Halli R C. Histochemical Analysis of Acid-phosphatase Activity Incident to Orthodontic Tooth Movement in Albino Rats - An Experimental Study. WebmedCentral ORTHODONTICS 2011;2(3):WMC001653

Source(s) of Funding:
Nil

Competing Interests:
None
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Abstract

Aims: The present study was designed to evaluate the Acid-phosphatase activity before and during orthodontic tooth movement in Albino rats to correlate histochemical changes with histological changes.

Methods: 12 healthy Albino rats were selected and divided into two groups for histological (Group 1) and histochemical (Group 2) analysis with one rat in group 1 and three rats in group 2 as control. The rats were observed in two phases of 24 hours and 72 hours and then subjected to the respective analysis.

Results: Histological analysis revealed no changes in 24 hours group and irregular margins with Howships lacunae formation in which osteoclasts were seen in 72 hours group. Histochemical analysis revealed increased activity of acid-phosphatase in the periodontal ligament which was randomly distributed in 24 hours group and increased activity of acid-phosphatase in the periodontal ligament which was randomly distributed in 72 hours group.

Conclusion: It was observed that histochemical changes appear as early as 24 hours with intensely positive reaction to acid-phosphatase. Overall analysis reveal that histochemical changes precedes the histological changes.

Introduction

Bone turnover during Orthodontic tooth movement has typically been described as balanced process; which allows tooth to move and also maintains the integrity of the alveolus. This bone remodelling involves a series of events in the alveolar bone and periodontal ligaments.

The study on the activity of acid and alkaline phosphatase in the periodontal tissue, was first published by Takimoto and Mori after insertion of rubber dam between first and second molars in rats (Takimoto, Mori 1968). Enzyme acid -phosphates is seen in relation to bone resorption. High Acid - phosphatase activity is observed in Osteoclast. The present investigation was done to study the activity of enzyme acid-phosphatase, as an indicator of bone resorption. In addition the histological changes occurring during orthodontic tooth movement were compared to histochemical changes.

Materials and Methods

This study was carried out at Department of Orthodontics at our institution. The ethical committee approval was obtained to conduct the study at our institution. The experimental animal chosen were Albino rats. Both male and female rats each weighing 140 + -20 grams were used. They were divided into two main groups according to the nature of the study to be carried out -

1) Animals used for Histological study.
2) Animals used for Histochemical study.

1) Animals used for Histological study were further subdivided into 3 groups-
   i- Control group-which included 1 rat where no orthodontic treatment was given.
   ii- Group A-included 1 rat , where an orthodontic appliance was fixed and the animal was kept under observation for 24 hours.
   iii- Group B-included 1 rat ,where an orthodontic appliance was fixed and the animal was kept under observation for 72 hours.

2) Animals used for Histochemical study were also divided into 3 groups-
   i- Control group- included 3 rats where no orthodontic treatment was given.
   ii- Group A-included 3 rats, where an orthodontic appliance was fixed and the animal was kept under observation for 24 hours.
   iii- Group B-included 3 rats , where an orthodontic appliance was fixed and the animal was kept under observation for 72 hours.

Mandibular central incisors were selected to fix the orthodontic appliance. They were selected because of their greater cervico-incisal length and better accessibility for preparation and placement of the appliance. The appliance consisted of bands along with eyelets welded in a vertical direction along the long axis of two Mandibular incisors .The active component of the appliance consisted of two vertical loops with two helical coils prepared out of 0.014
Each rat was anesthetized with solvent ether. When the rat was completely under the effect of the aesthetic agent the jaw reflex was abolished, the appliance was then fixed.

Euthanasia and specimen removal -
To obtain the tissue a lethal dose of ether, was given to the animal. Then the portion of mandible with the roots was dissected.

For Histochemical study nearly all enzymes disappear from the tissue quickly and progressively after removal of tissue from the body. So tissue was prepared in such a way so as to preserve enzymes as completely as possible and to retain its original sites.

The procedure adopted in this study for Histochemical technique was as follows.
1) Embedding-The dissected tissue from rats mandible was embedded in the embedding medium for frozen tissue specimens. The tissue TEC,OCT. Compound was used which was then immediately frozen at -70 degrees centigrade temperature in hexane cooled with carbon dioxide. Rapid cooling prevents any crystal formation in the tissue.
2) Sections-The blocks thus prepared were cut in cryomicrotome adjusted to obtain sections of 20 microns in thickness.
3) Sections were then fixed in acetone at room temperature for 30-40 seconds.
4) Staining-Acid-phosphatase of most tissues occur inside lysosomes. These sub cellular structure keep enzyme behind a relatively impermeable membrane so that very little activity can be demonstrated in intact tissues. At optimal pH the membrane itself becomes unstable and alters its permeability. Fixation renders the membrane totally permeable.

The method used for staining was- Gomori Lead phosphate method. All the solutions required for this method were freshly prepared, to have a good result. The sections for histological study were decalcified and stained with Eosine and Hematoxyline.

Results and Observations

The observations were -Clinical, Histological and Histochemical.
Clinically there was no marked inflammation of gingiva or other surrounding soft tissues in any of the rats in group A , group B and control group. 24 hours after the insertion of the appliance approximately 1.0 to 1.5 mm. of tooth separation was noted. 72 hours (group B) after the insertion of appliance approximately 3 mm. of tooth separation was observed.

Histologically the sections taken from the animals of control group showed normal bone dentin and periodontal ligament in their normal alignment. The sections taken from animals having undergone 24 hours (group A) of orthodontic tooth movement showed no definite change indicating bone resorption, whereas 72 hours (group B) of orthodontic tooth movement showed irregular margins with Howships lacunae formation in which osteoclasts were seen. Osteoclast of both varieties-mononuclear and multinuclear were observed (fig 2).

Histochemical analysis by Gomori Lead phosphated method showed acid -phosphatase activity as brown to black deposits.

The control group showed very few cells with acid -phosphatase activity randomly distributed in the periodontal ligament and alveolar bone. This minimal activity may be because of the normal bone remodelling process, which is going on throughout life. Group A (24 hours.) showed definitely increased activity of acid-phosphatase in the periodontal ligament which was randomly distributed (fig 3). Group B (72 hours.) increased activity of acid-phosphatase in the periodontal ligament which was randomly distributed (fig 4 )

Discussion

Enzyme acid-phosphatase activity is seen in relation to bone resorption. Acid -phosphatase is a lysosomal enzyme which has high activity in bone resorbing cells such as Osteoclast and macrophages. Acid phosphatase is less widely distributed than alkaline counterpart (Asma A A A, Rohaya M A W et al 2008). The optimum pH lies in the range of 5 to 6. Osteoclast are large cells. They may be mononuclear or multinuclear and have acidic cytoplasm. The resorption of bone requires both collagen and mineral to be dissolved. An acidic pH could account for dissolving the mineral. The enzymes of lysosomes could account for breakdown of the collagen. Histochemical localization of intracellular acid phosphatase is generally more discrete than that of alkaline phosphatase because it is localized mainly in specific membrane bound organelles the lysosomes.

In this study, clinically 24hours after the insertion of the appliance approximately 1.0 to 1.5 mm. of tooth separation and after 72 hours approximately 3 mm. of tooth separation was noted.

Normally clinically it is not possible to achieve 1 to 3 mm. of separation in such a small time span in human teeth ,with light continuous force. But in albino rat ,it may be because of the fast growth rate of lower
incisors (Rowett HCQ 1968). They grow approximately 2.8 mm. per week (Buck D L, Church D H 1972). The supportive tissues of rat incisors may be in proliferative stage due to continuous high -rate of eruption. Histologically in control group normal histological structure of bone dentin and periodontal ligament was seen .In group A (24 hours.) there was no definite change indicating bone resorption .In Group B (72 hours.) on pressure side irregular bone margins with Osteoclast in Howships Lacunae were seen.

In a study carried out by Takimoto and Mori in 1968 on 60 winstar strain rats the same finding was noted at 24 hrs (Takimoto, Mori 1968). Histologically they noted secondary bone formation on tension site at day 5. However in present study bone formation was not seen as the observations were done only for 3 days. A detail histological study of human tooth movement was done by Buck D L and Church D H in 1972. 70+-7grams of force was applied. In their study Histologically 7days after application of force compression of periodontal ligament space with undermining resorption was seen. Localized compression of periodontal ligament and frontal resorption was seen at day 14th . Fibre and cell reorganisation was principle finding at day 28. However in the present study, Histologically compression of periodontal ligament and irregular border of alveolar bone with few Osteoclast in lacuna were seen on the pressure side at day 3. This might be because of fast growth rat of lower incisors.

Histochemically, control group showed very few cells with acid-phosphatase activity randomly distributed in periodontal ligament and bone. This might indicate normal bone turn over. Group A (24 hours) showed minimal activity of acid-phosphatase randomly distributed. Where as group B(72 hours) showed localized intensely positive reaction to enzyme Acid-phosphatase . The same finding were seen by Lilja, Lindskog and Hammarstrom in 1983 (Lilja, Lindskog et al 1983). They studied enzymes associated with bone resorption and tissue damage in 10 rats. In the control group few cells with acid-phosphatase activity were seen randomly distributed . The enzyme activity was increased compared to control after 10 hrs. of Orthodontic force application. In addition they concluded that gradual increase in the activity of enzyme Acid-phosphatase was seen for both low and high force application. In 1993 Keeling, Stephen et al studied Acid and Alkaline phosphatase changes in serum and alveolar bone during Orthodontic tooth movement in adult male rats. An early wave of resorption (3-5 days) followed by its reversal (5-7days) and a late wave of formation (7-14 days)was seen in the alveolar tissues (Keeling D, Stephen et al 1993). The present study included changes in Acid-phosphatase levels up to 3 days which correlates with early wave of resorption.

Alkaline phosphatase has been found to be synthesised and secreted by the osteoblast cells during bone formation. Batra P, Kharbanda O P et al. in 2008 have studied Alkaline phosphatase activity in gingival cervicular fluid during canine retraction. Their result showed significant change in alkaline phosphatase activity on seventh, fourteenth and twenty first day on both mesial and distal aspect of the canine. The peak in enzyme activity occurred on fourteenth day (Batra P, Kharbanda O P et al. 2006). It can be concluded that the Histochemical changes precedes the histological changes, hence the importance of Histochemical study.

Conclusion

The following conclusions can be drawn from the present study.

1) Variation in acid phosphatase activity was noted before and during the orthodontic treatment and the activity gradually increased.
2) When no orthodontic force was applied Acid-phosphates activity was very minimal, may be because of the normal remodelling process occurring in the bone.
3) After 24 hours of orthodontic force application there was no definite change indicating bone resorption Histologically but Histochemically it showed definite increase in Acid-phosphatase activity which was randomly distributed in the alveolar bone and periodontal ligament.
4) After 72 hours of orthodontic force application a definite bone resorption pattern was seen Histologically with irregular bone margins and osteoclasts within Howships lacuna on pressure side. Histochemically there was intensely positive reaction to Acid-phosphatase. The enzyme activity was localized in periodontal ligament.

The Histochemical study is a modern advancement in tissue turnover technique. It was observed that Histochemical changes appear as early as 24 hours. So it can be concluded that the Histochemical changes precedes the histological changes.

References

orthodontic tooth movement: Canine retraction stage.

Figure Legends

Fig 1. Photograph showing 3 m.m. separation of incisors after 72 hours.
Fig 2. Photomicrograph of decalcified section of bone with osteoclasts after 72 hours. H. and E. (X 100 )
Fig 3. Photomicrograph of Undecalcified section showing minimal Acid-phosphatase activity Randomly distributed in the Periodontal ligament, after 24 hours (x 12.5 ).
Fig 4. Photomicrograph of Undecalcified section showing localized intense Acid phosphatase Activity in the Periodontal ligament after 72 hours.( X 12.5 )
Illustrations

Illustration 1

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Illustration 2

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