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Histological Evaluation of Tissues Using a Bone Inducing Substance in Cases of Micro-Screw Implant "An Experimental In Vivo Study"

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الخلاصة

الأهداف: تهدف الدراسة الى تحديد امكانية استخدام عقار الالندرونيت صوديوم لزيادة فعالية وكمية الخلايا المكونة للعظم حول الغرسة التقويمية. **المواد وطرائق العمل:** أستخدم في هذه الدراسة ثمانية وأربعون غرسه تقويمية، اثنا عشر أرنب حقلي. قسمت الأرانب إلى مجموعتين رئيستين، مجموعة خضعت لعلاج بالالندرونيت صوديوم وأخرى بدون علاج للمقارنة، قسمت كل مجموعة إلى ثلاث مجاميع فرعية حسب الفترة المقررة لامكانية التئام العظم وهي اسبوعين، اربعة اسابيع وست اسابيع، تم غرس ثمان غرسات تقويمية في كل المجاميع الفرعية التي خضعت للعلاج وست زرعات في المجاميع الفرعية المقارنة، تم فحص النسيج العظمي المواجه للغرسات التقويمية نسيجيا لكشف كمية الخلايا الهاضمة والمكونة للعظم. **النتائج:** أظهرت نتائج الدراسة اختلافات معنوية لكمية الخلايا المكونة للعظم بين فترات الالتئام المحددة للمجاميع الفرعية المعالجة، كذلك الحال عند مقارنة المجاميع المعالجة بالمقارنة في فترة الاسبوعين المخصصة للالتئام. اما بالنسبة للخلايا الهاضمة فقد لوحظ اختلاف معنوية بين الفترات المحددة للالتئام لكلا المجموعتين المعالجة والمقارنة.**الاستنتاجات:** من نتاج البحث يمكن القول انه لا يوجد اختلاف في التفاعل الالتئامي للأنسجة عند المقارنة بين المجموعة المعالجة بالمجموعة المقارنة. وعليه لربما من الافضل اطالة فترة استخدام العقار لامكانية حدوث تغيير واضح على العظم.

ABSTRACT

Aims: To evaluate the effects of systemically administered Alendronate sodium on osteoclastic activity and osteoblast accumulation. **Materials and Methods**: Fourty eight micro-screw implant, twelve adult rabbits were used in this study, the rabbits divided into two main groups, treated and control group, which further subdivided into three subgroups according to the healing periods after 2 weeks, 4 weeks and 6 weeks. All animal were treated with alendronate sodium. Eight micro-screw implants instilled in tibiaes of each tested subgroup and six instilled in tibiaes of control subgroups, the tissue facing the micro-screw implant subjected to histological evaluation for the tested subgroups and control subgroups including the number of active osteoblast and osteoclast. **Results:** A significant differences between subgroups 0WT, 2WT and 4WT, were more prominent for 2WT and 4WT subgroups from 0WT subgroups which showed score (+++), further no significant difference for control subgroups were all showed score (+++) for osteoblastic activity. For the active osteoclast a significant differences detected between subgroups of experimental and control sample, were score (+) noticed in the 0WT subgroups, score (+) in 2 readings and (++) in 6 readings of4WT subgroups and score (+++) for 4WT subgroups with no significant difference between experimental and control samples in 2 weeks, 4 weeks and 6 weeks healing periods .**Conclusions**: According to the result of histological evaluation, no estimated differences in the bone remodeling process had been detected between treated and the control group, which may need longer period of drug administration to probably induce effect on bone.

Key words: Histology, Micro-screw implant, Bone inducing substance**.**

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INTRODUCTION

Bisphosphonates are now the most widely used drugs for the treatment of some metabolic bone diseases, such as Paget's disease, osteogenesis imperfecta, fibrous dysplasia,Gaucher's disease, malignant hypercalcemia, and osteoporosis. Bisphosphonates can bind to hydroxyapatite crystals in a mineralized bone matrix and make the bone more resistant to osteo-

clasts, inhibit differentiation of bone marrow precursors into osteoclasts, inhibit osteoclast function by interfering with the mevalonate pathway of cholesterol biosynthesis, and induce apoptosis of osteclasts.[1] In recent years, it has become known that bisphosphonates not only restrict osteoclastic activity, but also show osteo-conductive and osteo-inductive effects by increasing osteoblastic activity.[2]

Micro-screw implants have grown in popularity as an important tool to facilitate maximum anchorage without the use of extra-oral appliances.[3,4] Increasing knowledge about the healing phase is important for an understanding of factors that are required to achieve implant-bone integration and to identify the optimal time of initial loading. The aim of this study was to evaluate the effects of systemically administered Alendronate sodium on osteoclastic activity and osteoblast accumulation.

MATERIALS AND METHODS

Fourty eight commercially available self-drilling titanium micro-screw implants (Abso-anchor, Dentos Inc, Daegu, Korea), twelve adult female rabbits and fourteen alendronate sodium ampules for intra muscular injection were used in this study. Each micro-screw implant was selected from the same series, with the same length, diameter and similar shape. measuring 1.3 mm in diameter and 5 mm in length. The rabbits were 12 months old (each weighting 2 kg) and the drug Pharmacologic class: Bisphosphonate, Therapeutic class: Bone-resorption inhibitor, its Action is impeding bone resorption by inhibiting osteoclastic activity, absorbing calcium phosphate crystal in bone, and directly blocking dissolution of hydroxyl apatite crystal of bone.

All micro-screws and rabbits were divided into two groups: the first untreated group(control group) is the plane group (p) which further subdivided into three subgroups, which are, the immediate loading plane subgroup (0WP), 2-week healing plane subgroup (2WP) and 4-week healing plane subgroup (4WP) while the second group is the treated group (T) which also subdivided into three subgroups, the immediate load treated subgroup(0WT), 2-week healing treated subgroup (2WT) and 4 week treated subgroup (4WT). The drug given to each rabbit intra muscularly (0.3 ml, using the insulin syringe) for fourteen days before Micro-screw implant instillation (which is just one of the multiple courses recommended, to human, by the manufacturer, Diamond Pharma Damacus-Syria under license of ABC Farmaceutici-Torino-Italy). There were 8 micro-screws in each subgroup six of them tested and two cancelled due to failure like fracture of screw during driving and some shows bone crack at penetration of screw. There were two rabbit in each of the six subgroups, these six subgroups may encounter the three pathological periods of bone healing after the instillation of the micro-screw implant, that is, the traumatic period, granulation period, and callus period, respectively.[5] The micro-screw implant were instilled in the tibiae of each animal, the tissue facing the micro-screw implant subjected to histological evaluation for the tested subgroups and control subgroups including the number of active osteoblast and osteoclast were scored as + (1-10), ++ (11-20),or +++ (>20).[1]After washing with distilled water, these ctions were counterstained with hematoxylin and Eosin in 40 times magnification.

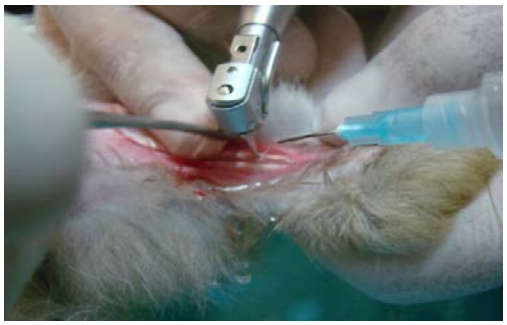
Surgical Procedures:

All surgeries were performed under sterile conditions in an animal operation room. The animals were anesthetized intramuscularly with a combination of ketamine + xylazine (44+7 mg/kg of body weight). The local nerve supplies of the internal surface of the tibia were further blocked with 0.5 ml of 2% Lidocaine (Figure 1). The tibiae body was exposed by incisions through the skin, fascia, and periosteum (Figures 2,3). The cortical bone of the preparation sites was penetrated using a 0.6mm-diameter guide drill under profuse irrigation (Figure 4).

Figure (1): The local nerve further anaesthetized fascia. with 0.5 ml 2% lidocainperiosteum

Figure (2): Incision through the skin,

Figure (3): Exposure of surgical site



Figure(4): Guid drill under perfused irrigation

After pilot drilling, the Micro-screw implants were placed using a manual driver (Figures 5 and 6). All Micro-screw implants were allowed to penetrate the first cortical layer and going through the woven bone only (not penetrating the opposing cortical plate). The loading involved nickel-titanium closed-coil springs (Denturum) were applied to the coronal portion of the Micro-screw implants with 100g of force using tension gauge (Anthogyr company, France) (Figure 7 and 8). The mucoperiosteum and muscle were sutured in separate layers using absorbable sutures (Figure 9).

Figure (5): Manual drilling of the micro-screw implant

Figure (6): Micro-screw implants in its sites

Figure (7): loading the micro-screw implant

Figure (8): Micro-screw implant loaded

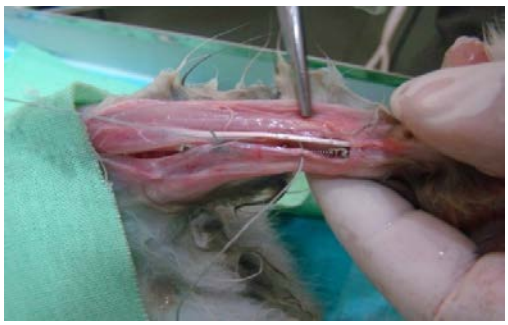


Figure (9): Suturing the tissue layers with absorbable suture

Figure (10): Tibias tissue of experimental site fixation

The histological testing involves the following:

The tibias at the experimental site were taken immediately after the animals were killed by slaughtering. After tissue fixation (Figure10),embedding, sectioning and de-calcification then slicing with a microtome YD-150A, and staining with hematoxylin and eosin (H&E) stain of the decalcified bone slices and slide preparation (Figure 11,12) followed by observation under anoptical microscope, Motic Lense EA 40x magnification and the histological site captured with specialized camera for this purpose (assembled with the microscope) and the captured photos managed with A shampoo photo commander 7 programme (Figure 13).

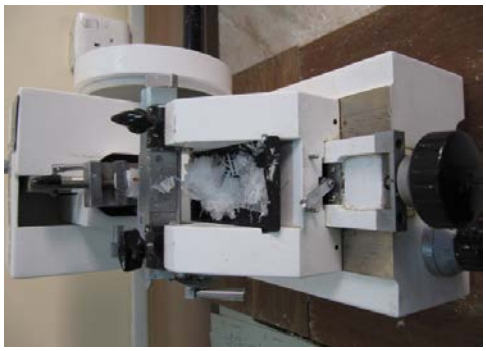


Figure (11): Slicing the wax blocks of histological slices stained Part of tibiae bone

Figure (12): Prepared slides of histolog-with Haematoxyline and Eosin

Figure (13): Capturing the histological sectioning

In the 0WT and 0WP subgroups the bone at implant site were evaluated histo-logically for the number of active osteo-clast and active osteoblast after 2 week healing period.The same thing for 2WT and 2WP subgroups also tested in the same manner histologically but after 4 weeks healing period. For the 4WT and 4WP subgroups the histological evaluation did after 6 weeks healing period.

Statistical analysis:

The data were analyzed by using SPSS for Windows(version 11.0, SPSS), de-scriptive statistic and Mann-Whitney test

done to compare differences between the three subgroups while the Kruskal-Wallis test to compare differences between the tested and control subgroups. The level of significance was set at $P<0.05$**.**

RESULTS

Mild inflammation and swelling after the surgery in each tibiae, two measurements were cancelled as a result of abscess around the micro-screw implant. For osteoblast activity evaluation, the statistical analysis showed significant differences between subgroups 0WT, 2WT, and 4WT. Subgroups 2WT and 4WT were more prominent from 0WT subgroup which showed score (+++) compared to 0WT subgroup which showed scores (+) in three reading and (++) in five reading, further no significant difference for control subgroups were all showed score (+++).

The study also showed a significant difference between experimental and control subgroups for 2 weeks healing period, score (+++) for control subgroups and score (+, ++) for treated subgroup, but no differences detected for 4 and 6 weeks healing periods Table (1).

Table (1): Represents descriptive statistic and statistical analysis of the osteoblast activity

Experimental Subgroups	**0WT**	**2WT**	**4WT**	**Kruskal-Wallis Test**
+	3	0	0	Chi-Square = 22.135
++	5	0	0	P = 0.000
+++	0	8	8	Significant
Control Subgroups	**0WP**	**2WP**	**4WP**	**Kruskal-Wallis Test**
+	0	0	0	Chi-Square = 0.000
++	0	0	0	P = 1.000
+++	6	6	6	Not Significant
Mann-Whitney Test	U = 0.000 P = 0.001 S	U = 24.00 P = 1.00 NS	U = 24.00 P = 1.000 NS	

+ : Osteoblast number (1-10), ++ : Osteoblast number (11-20), +++ : Osteoblast number >20

For the active osteoclast a significant differences detected for experimental and control subgroup, within subgroups of treated and control samples, for the three healing periods, were score (+) noticed in the 0WT subgroups, score (+) in 2 readings and (++) in 6 readings of 4WT subgroups and score (+++) for 4WT subgroups. No significant difference between experimental and control subgroups in 2 weeks, 4 weeks and 6 weeks healing periods Table (2).

Table (2): Represent descriptive statistic and statistical analysis of the osteoclast activity

Experimental Subgroups	**0WT**	**2WT**	**4WT**	**Kruskal-Wallis Test**
+	8	0	2	Chi-Square =20.810
++	0	0	6	P = 0.000
+++	0	8	0	Significant
Control Subgroups	**0WP**	**2WP**	**4WP**	**Kruskal-Wallis Test**
+	6	0	0	Chi-Square = 17.000
++	0	0	6	P = 0.000
+++	0	6	0	Not Significant
Mann-Whitney Test	U = 24.000 P = 1.000 NS	U = 24.00 P = 1.000 NS	U = 18.000 P = 0.202 NS	

+ : Osteoclast number (1-10), ++ : Osteoclast number (11-20), +++ : Osteoclast number >20

DISCUSSION

The use of skeletally-anchored screws has generated widespread interest in orthodontic therapy, since the anchorage can be designed without the patient's cooperation. Temporary cortical anchorage can be recommended for a wide range of indications, nearly all of the micro-implants,

pins and bone screws currently in clinical use have one thing in common a part of the thread that must be anchored cortically.[3]How well a screw fits in the bone? essentially depends on the contact zone between bone and metal. The greater the amount of bone found resting against the turns, the better the screw fits.[6]

Micro-movements between the screw and bone are prevented by the rigid attachment application and the resulting possible rotation of the adapter wire around the screw axis, since micro-movements have a sustained negative effect on bone formation and Osseo integration in the area of the screw turns. Thus the problem concerning an individual implant's rotational stability and its potentially difficult osseointegration is minimized when using this anchorage technique. Opinions regarding cortical healing and clinical suitability vary on how necessary pre-drilling is prior to inserting the screw. Vangsness *etal*.[7] investigating self-tapping and non-self-tapping screws, found stress cracks in the bone close to where the screws had been turned.

In this study the statistical analysis showed that osteoclastic activity is the same for experimental and control group in the three healing periods (after 2 weeks, 4 weeks and 6 weeks) so no significant effect of the drugs given could be noticed and this is disagree with the postulation of Fleisch[8] in that Bisphosphonates are potent inhibitors of bone resorption that not only inhibit dissolution of hydroxyapatite crystals, but also affect osteoclast metabolism and function. Lee *et al*,[9] investigated the effects of first-generation bisphosphonates (etidronate) histochemically and showed that the number of osteoclasts and the relapse ratio were less in the etidronate group than in the control group. They stated that, because the bisphosphonates decrease the number of osteoclasts, the potential for relapse after mechanical expansion of the suture is reduced and this is also disagree with the result of this study, which may be postulated by the approach followed in this experimental study in that the rabbits given only one course of alendronate and the ordinary schedule of the drug according to the manufacture instruction is a multiple course for 6 months period as denoted in material and method.

The above postulation probably be the same reason for the result of the osteoblastic activity as it is estimated in this study, were it is not so prominent to be induced by this drug as shown in number of studies that bisphosphonates not only inhibit osteoclast function but also induce osteoblastic activity.[10, 11]Some studies have concentrated on locally applying bisphosphonates to implant surfaces and then measuring the bone response. Statistically significant increases in bone density and bone formation occurred with the alendronate-coated implants.[12]A complete understanding of this drug class and the effects on long-term implant osseointegration in humans will require further study add to the need for long treatment period following the instructions proposed by the manufactures of drug to ensure possibility of drug effect on the bones.

Significant differences noticed between different healing periods especially for experimental subgroups which could be the result of ordinary healing procedure were active osteoclast became more evident in the 4WT and 4WP subgroups. A new bone formation and active osteoblast were more evident in 6WT and 6WP subgroups see figures(14, 15 and 16) that representing the three different healing periods with an important note that the stability of all Micro-screw implant in all healing periods was acceptable as tested by using the Periotest machine (Medizin technik Gulden e.k. Eschenweg 3, 64397 Modautal, Germany) and this approach is adjunctive to the histological evaluation, where the remodeling process more evident in the medullary bone and the acceptable stability may be from the cortical bone.

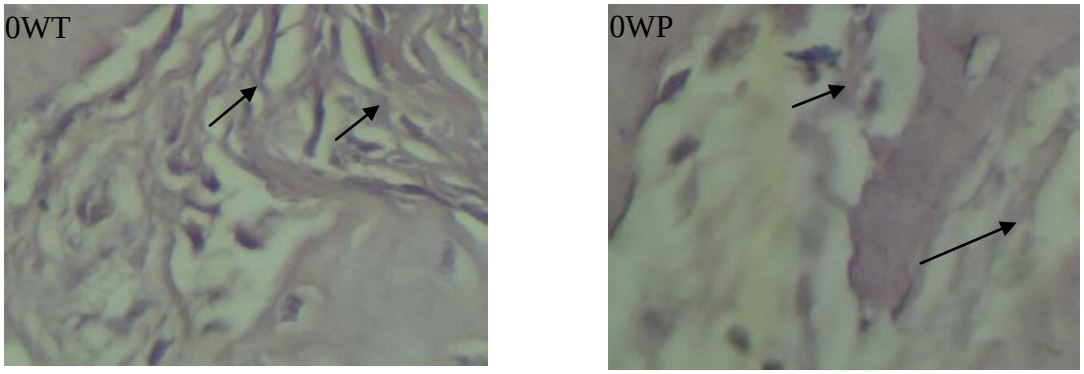


Figure (14): Represent two week healing period haematoxyline and eosin staining (40x light microscope) indicates the location of disassociation bone tissue facing the implant during the processing procedure of decalcification as illustrated by the arrows which varying in form according to the site and direction of sectioning

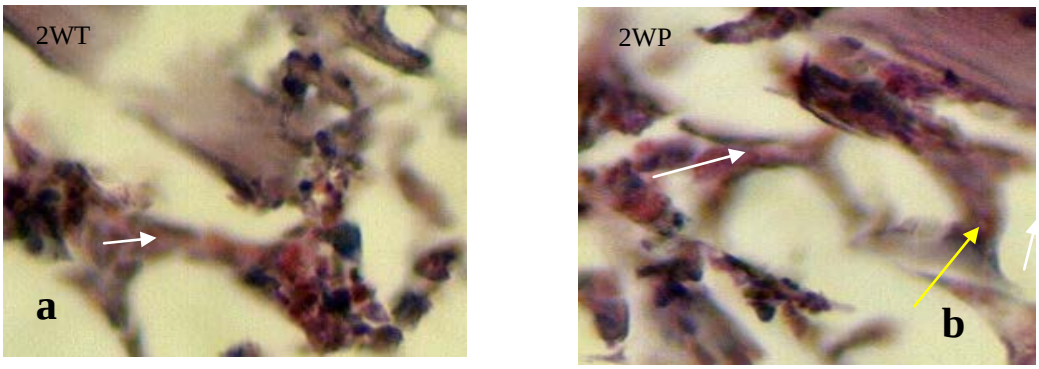


Figure (15): Represent 4 weeks healing period showing the bone remodeling process with Haematoxyline and Eosin staining under magnification (40 xs light microscope). (a) The yellow arrow denote to the osteoblast aggregation and proliferation that will start and direct the release of osteoid matrix. (b) The white arrow denote to new bone formed (osteoid)
Note: the problem encountered here that we did not have bone microtome so all the samples are decalcified the sliced for histological evaluation

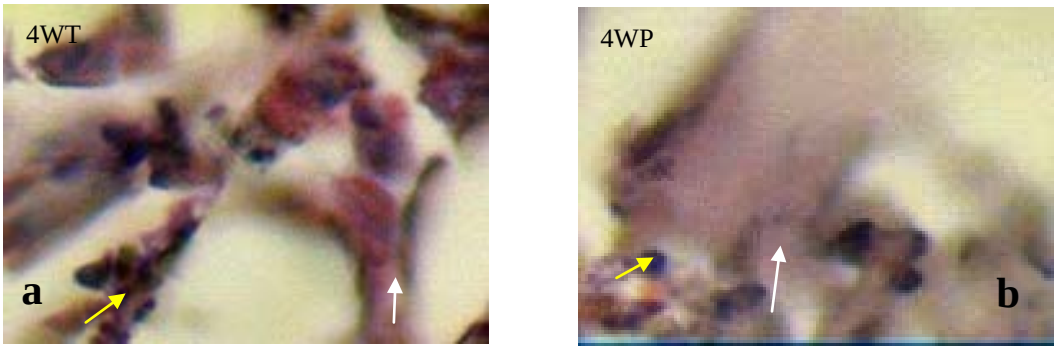


Figure (16): Represent 6 weeks healing period showing the bone remodeling process with haematoxyline and eosin staining under magnification (40 xs light microscope). (a) Yellow arrow represents the osteoblast. (b) Osteoid matix.
Note: the problem encountered here that we did not have bone microtome so all the samples are decalcified the sliced for histological evaluation

CONCLUSION

According to the result of histological evaluation, no estimated differences in the bone remodeling process had been detected between treated and the control group, which may need longer period of drug administration to probably induce effect on bone that is discriminative.

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