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Histopathological evaluation of Tilduronate on healing of femoral bone in dogs

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ABSTRACT

The study was designed to evaluate the clinical and histopathological effects produced by the systemic administration of Tildronate (Bisphosphenates compound), on the healing of experimentally induced femoral bone fracture in twelve adult dogs, at which the fractures were fixed by Steinman intramedullary pins. The dogs were divided randomly into two groups, six for each. Following induction of femoral bone fracture and intramedullar fixation, the dogs in the treated group were treated with tiludronic acid at 2 mg / kg body weight, subcutaneously, twice weekly, for eight consecutive weeks. In control group the fracture was fixed by steinmam's intramedullary pin and was left to repair spontaneously without further medical treatment . All dogs were followed for two months by routine clinical inspection and the animals of other group were scarified at; 21, 45, and 60 days, respectively, to collect specimens from the fracture site to prior histopathological examination. The results revealed that the potential active effect of Tiludronate on the course of fracture healing was to minimize the activity and life span of osteoclast cells at the fracture site, contributing to a pause in bone remodeling. The influence of this effect was demonstrated by the delay in the time taken by the bone to regain its normal shape in the treated community, compared with the time taken by the control group.

1. Introduction

The physiological processes of fracture healing have been the focus of an active investigation for many years. Unlike other tissues that heal by scar tissue development, bone heals by regenerating new bones. [1-2]. Until recently, the main progress has been in the surgical procedures, which have allowed solid stabilization of the fractured segments. Attempts to develop drugs to promote bone formation have not yet been effective, although bone-forming growth factors have been identified, such as the bone morphogentic proteins (BMPs), transforming growth factors (TGFs), fibroblast growth factors (FGFs), and others, hopes that we will soon make use of their anabolic properties [3].

The healing of fractures is divided into t hree phases: inflammatory, reparative, and remodeling.Following the initial inflammation, both intramembrane ossification and endochondral ossification form new bones: these processes are primarily mediated by osteoblasts [4-5]. This phase is followed by an extended period of remodeling involving osteoclasts that resorb the new woven bone and osteoblasts that replace this matrix with lamellar bone [6]. As with homeostatic remodeling, the important functional outcome of the remodeling phase of fracture healing is the restoration of mechanical strength and stability[4-5].

The process of bone and fracture repair consist of an anabolic (bone forming) response and a catabolic (bone resorbing) response. In the absence of an anabolic response, anti-catabolic treatment alone does not lead to union in a rat femoral critical defect model[7]. Treatment with bisphosphonate (BP) my require anabolic conjunctive therapy to ensure enhanced successful repair [7-8] .

Investigators have addressed the positive or negative influence of bone resorption inhibitors on fracture healing. Rather, emphasis has been largely on the inhibition of fracture incidence. However, with the wide use of the BP, more recently, attention has focused on whether these drugs are, in fact, deleterious to fracture healing. Therefore, experiments in various animals are now available, which investigate BP effects on the healing of fractures[3].

Over the years, there have been concerns about whether or not BP interferes with the fracture healing. Because they suppress bone remodeling, one might expect that BP interfere with fracture repair. In a growing rat model using incardinate, it had been reported that BP treatment resulted in a larger fracture callus and delayed maturation of the fracture[9]. Alendronate treatment also suppressed remodeling of the fracture callus in ovariectomized rats[10]. These changes may be secondary to inhibition of bone resorption because bone formation and resorption are intimately linked. Conversely, there are reassuring reports on this topic that show fracture callus remodeling is not a problem in several animal models unless very high doses of BP are used [11-12]. In contrast to these concerns, there are now several reports suggesting that BP may actually enhance fracture repair, probably by stabilizing the fracture callus[7]. The important potential applications of BP in orthopedics, including protection against loosening of prostheses [13], better integration of biomaterials and implants[14], improved healing in distraction osteogenesis [15]. The aim of the study was to find out the effects of tiludronate (tiludronic acid) on fracture healing in dog's femur.

2. Materials and Methods

2.1 Experimental Animals

The study included 12 young adult dogs from both sexes. The age and body weight mean±SE are 8.4±4.8 months and 6.2±2.0 kg, respectively. All the dogs were of local breed and were physically healthy. During the experiment the dogs housed and strictly supervised the Department of Veterinary Surgery and The riogenology, College of Veterinary Medicine, University of Mosul, in the animal's housing.

2.2.Surgical operation:

Animals were anesthetized with 15 mg/kg, of Ketamin (alfasan, woerden-Holland) and 5mg Xylazine (VMD,Belgum) were intramuscularly injected The right femora of all animals was experimentally fractured by wire saw in the mid-diaphysis and repaired using the standard aseptic surgical procedures with Stainman's intramedullary pins (2,5 to 5 mm diameter) [16].

2.3.Experimental Design:

Following surgery, the dogs were divided into two groups randomly and equally (6 dogs

for each group); treatment and control groups. treatment group received 2mg / kg of tiludronic acid, subcutaneously, twice a week for eight consecutive weeks until sacrificed (Tildren®;Tiludronic acid 50mg. By: CEVA SANTE ANIMAL, 33500 Libourne, Franrijik). However, the control group did not receive bisphosphonate medication. histopathological examination of all fractured femora was taken at (21,45 and 60) days respectively throughout the study course to all dogs.

2.4.Histopathological study:

Microscopic examinations of the bone healing were performed on all dogs at 21, 45 and60 days after treatment. Two dogs used for each period. An overdose of xylazine-ketamine mixture was used to euthanize & sacrifice the rats at every interval of time. The right femurs were harvested and stored for analyses.

2.5.Decalcification:

Buffered formalin 10% was a satisfactory fixative for femoral bone to 72 h [17]. Then the samples were washed with tap water for 24 hours & incubated using four altered decalcifying solutions: such as, 3% nitirc acid; 8% formic acid/hydrochloric acid; 10% EDTA (pH 7.4) and 5% nitric acid, after that samples were neutralized using 0.1% aqueous ammonia solution for 30 minutes. Further, decalcification was accomplished using continuous shaking [18]. Moreover, the daily change of decalcifying solutions as well as the exact timing of decalcification was noted down. The procedure of decalcification ended when bone was easily penetrated through by a needle without any force [19]. After modulation the specimens in paraffin, sequent slices of 5 µm thickness were performed. The histological slides were then stained by hematoxylin eosin [20].

3.Results and Discussion:

3.1 Treated group

The results of histological examination of the animals of the treated group, (21 days) after the fracture occurred, showed that the callus formed a mixture of fibrous tissue and early bone tissue. The appearance of this early callus was due to an attempt to speed up the construction of a bridge connecting the ends of the broken bone and indicating the activity of the osteoblast in addition to the rapid return of the processing (Fig.5) . This was confirmed by the researchers Li et. al.,(1999)[21]. when they reported that the administration of bisphosphonates leads to osteoblast activity. This results in a fast and thick development of the callus, which acts to connect the two sections of the fractured bone in a brief period of time, in addition to the existence of large numbers of acute inflammatory cells and the incidence of bone platelet necrosis.

After (45 days) of fracture, it was observed that the cartilage tissue of the vitreous type was formed, in addition to the dense fibrous tissue that completely connected the ends of the broken bone(Fig.6), as well as the presence of a large number of fully developed bone sacs, indicating that the fracture had undergone rapid healing stages as a result of the use of bisphosphonates(Fig7), and this is confirmed by the researchers Li et. al.,(2001)[22].

After 60 days of the fracture process , in addition to cellular debris and inflammatory cells, mature cartilage tissue of the vitreous type associated with the mature fibrous tissue was observed, as well as the presence of mature, non-necrotic bone sacs, but in a small way and this indicates that the process of bone restoration has begun to appear in this group(Fig.8), but very gradually, and this is the opposite of what was found in the control group, and that is what the researchers verified by Rodan and Fleisch , (1996)[23] and Morris and Einhorn , (2005)[24] when they said that

administering all kinds of bisphosphonates works to postpone the bone polishing and regeneration process in a timely manner to reduce the survival and functioning of osteoclasts.

3.2 Control group. .

The results of the histological examination of the first group of animals, (21 days) after the fracture, showed that a callus(fibrous cartilage) was formed(Fig.1), and this is the researchers confirmed by Arnoczky et. al.,(1985)[25] when they showed that cartilage tissue will form vitreous during a two-week fracture period, which builds a primary bridge between the two parts of the broken bone In an attempt to estimate The two parts can be fixed and the speed and smoothness of the tissue depends on the blood supply to the fracture area.

After 45 days of fracture, in addition to the presence of bone sacs between the bones, mature cartilage tissue formation was observed(Fig.2), and this is confirmed by the researchers(Stracher et. al.,1990)[26] when they stated that the appearance of the bone sacs is an indication of the external activity of the periosteum, which works to support the bonding of the two parts of the broken bone And this activity also depends primarily on the amount of blood supply to the area and the soft tissues present in the area(Fig.3).

In addition to the presence of vascular callus on parts of necrotic bone tissue, 60 days after fracture, cell debris and inflammatory cells were detected in the area under the bone vesicles(Fig.4), where the researcher indicated(Walter, 1981)[27] that these debris and inflammatory cells were removed by phagocytosis. Bone pieces are removed by the osteoclasts. The appearance of necrotic bones is an indication of the connection of the two parts of the broken bone completely and completely, and of the beginning of the process of restoring the fractured bone to its normal or near normal position, where the bone osteoclasts are re-refining and restoring the bone at this stage, and this is the same as stated by the researchers (James and Heckman, (1991)[28]; Enihorn, (1998)[29] and Kalfas, (2001)[30].

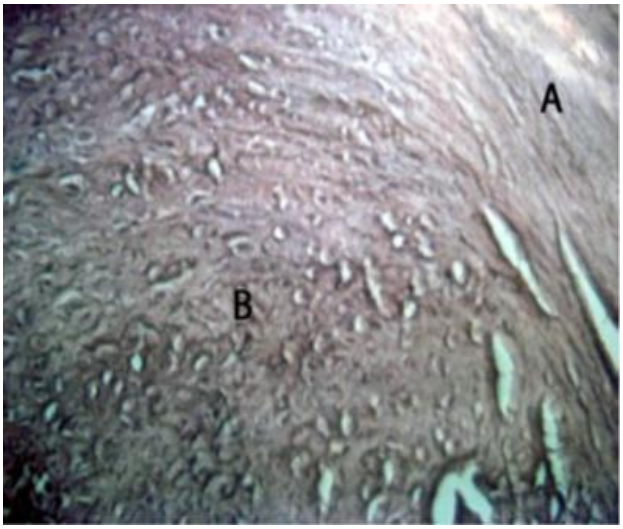

Fig. 1:Microscopical picture of Control group, 21 days post-operation, showing (A) Fibrous tissue (B) begin of cartilage callus formation. (H&E X100)

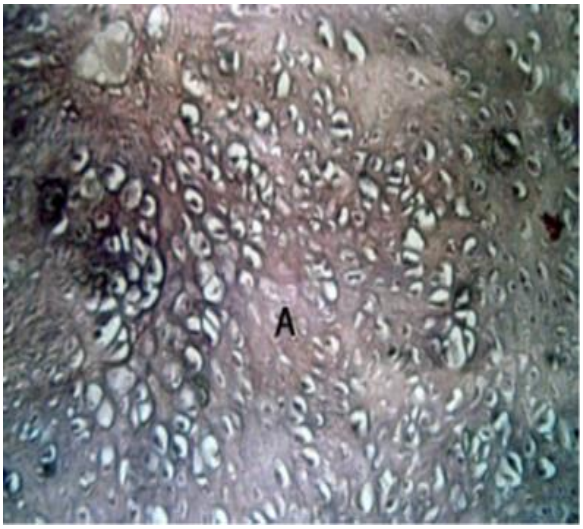

Fig. 2:Microscopical picture of Control group,45 days post-operation, showing (A) cartilaginous callus attached to the ends of the fractured bone (H&E X100)

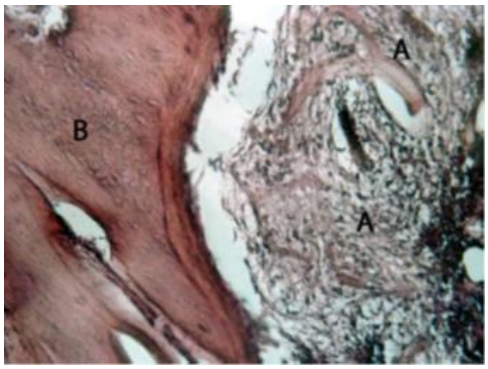

Fig. 3: Microscopical picture of Control group,45 days post-operation, showing (A) development of fibrous tissue between the bony sacs. (B) original bone sacs. (H&E X100)

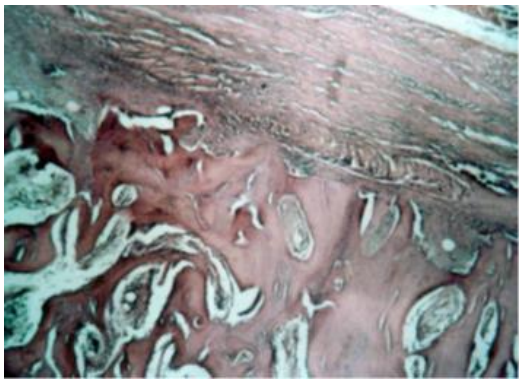

Fig. 4: Microscopical picture of Control group,60 days post-operation, showing, bony callus was formed attached to the fibrous portion of the periosteum. (H&E X100)

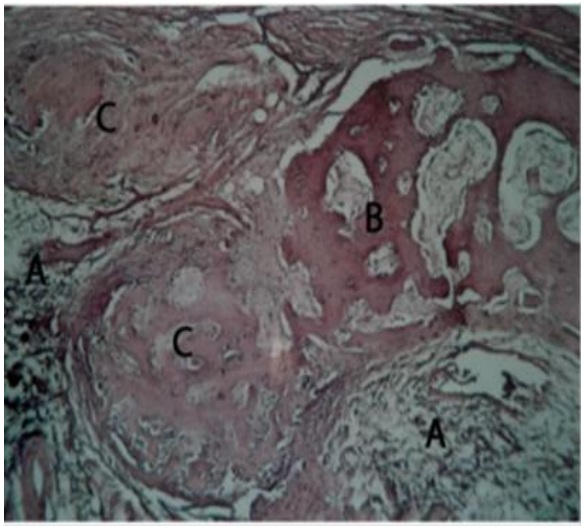

Fig. 5: Microscopical picture of Treated group, 21 days post-operation, showing (A)Fibrous tissue (B) Bone tissue (C) necrosis of the bony plates. (H&E X100)

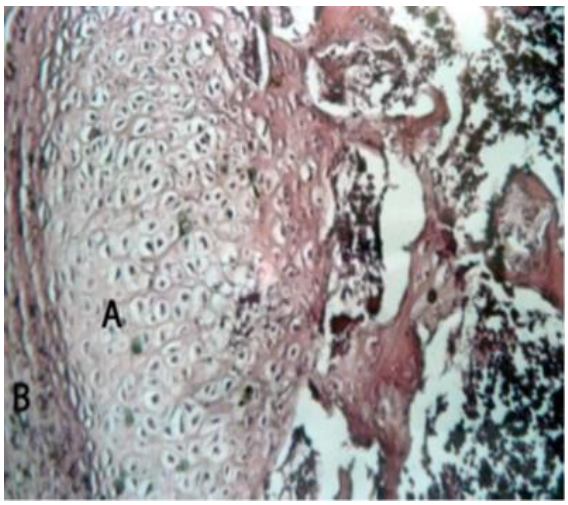

Fig. 6: Microscopical picture of Treated group, 45 days post-operation, showing (A) cartilage tissue of the hyaline cartilage (B) dense fibrous tissue. (H&E X100)

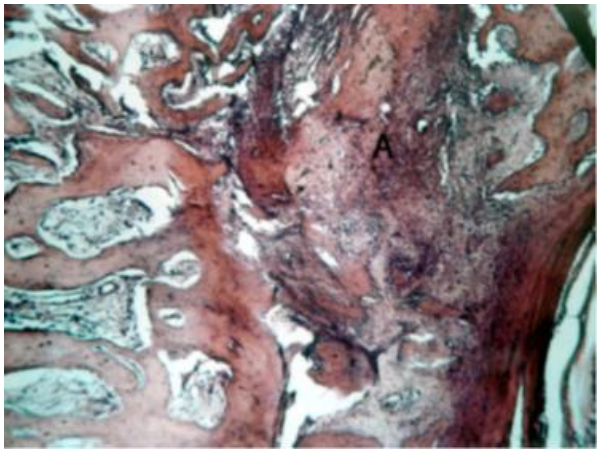

Fig. 7: Microscopical picture of Treated group, 45 days post-operation, showing (A) transformation of the callus into new-growing bone sacs . (H&E X100)

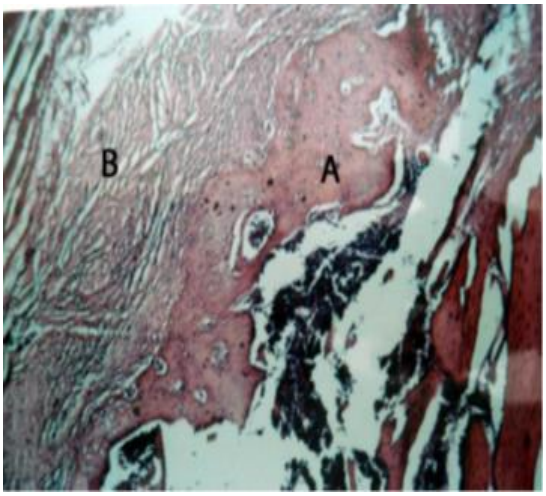

Fig. 8:Microscopical picture of Treated group, 60 days post-operation, showing (A) full-growing bone sacs (B) Fibrous tissue . (H&E X100)

Conclusion

Femoral fracture showed a good response to tiludronic acid treatment. The clinical course and histological finding demonstrated the value of using this bisphosphonate drug for promotion of fracture healing.

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التقييم النسجي –المرضي لأستخدام عقار التلدرونيت على التئام كسر عظم الفخذ المحدث تجريبيا في الكلاب

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الملخص

شملت الدراسة تقييم تأثير التغيرات السريرية والنسجية الناتجة عن استخدام التلدرين (مركبات البزفوسفونيت) من ناحية تأثيره على التئام الكسر في عظم الفخذ في الكلاب. حيث تم اجراء كسر تجريبي مستعرض في عمد عظم الفخذ لأثني عشر كلبا بالغا ومن كلا الجنسين، وثبت الكسر بعد ذلك باستخدام مسمار ستنمان للتثبيت الداخلي. قسمت حيوانات التجربة عشوائيا الى مجموعتين ضمت كل مجموعة ستة حيوانات مجموعة العلاج (العلاج بالتلدرين) تم اجراء الكسر ومن ثم التثبيت الداخلي وعولجت الحيوانات بجرعة (2ملغم/كغم)من وزن الجسم وتم الحقن تحت الجلد وبمعدل جرعتين اسبوعيا بعد اجراء الكسر ولمدة شهرين اما مجموعة السيطرة فترك فيها حدوث عملية الالتئام في الكسر التجريبي في عمد عظم الفخذ والذي ثبت بواسطة مسمار ستنمان دون تداخل علاجي . وتمت متابعة الحالات في مجموعتي العلاج والسيطرة سريريا ونسجيا ولمدة شهرين . حيث كانت المتابعة السريرية تجرى يوميا وخلال هذه الفترة تم قتل الحيوانات في الفترات (21، 60,45) يوما وبواقع حيوانين لكل فترة لغرض الفحص النسجي ودراسة التغييرات الناتجة من جراء اعطاء عقار التلدرين بالمقارنة مع مجموعة السيطرة. وتبين من نتائج هذه الدراسة :

أ- كان لاستخدام عقار التلدرين اثرا سريريا واضحا في تحسين الاداء الوظيفي للطرف المصابة في مجموعة العلاج مقارنة بمجموعة السيطرة.

ب- أدى حقن هذا العقار الى خفض نشاط ناقضات العظم وبالتالي ادى الى تأخير عملية ترميم العظم وعودته لوضعه الطبيعي مقارنة بمجموعة السيطرة.

ت- تسبب العلاج بالتلدرين الى حصول زيادة تكاثرية ونضوج سريع في الخلايا البانية للعظم مما ادى الى حدوث زيادة في تكوين الدشبذ مقارنة بمجموعة السيطرة .