



CLINICAL AND HISTOLOGICAL STUDY FOR THE INFLUENCE OF KETOROLAC ON BONE REPAIR IN RABBITS

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ABSTRACT

This study was designed to determine the effect of ketorolac on bone repair process in domestic rabbits. Forty adult domestic male rabbits were used in this study, weighing (1.036 ± 0.107) kg. They were divided randomly into two equal groups control group and Ketorolac group. Both groups undergoes surgical bone cavitation operation in femur bone via general anesthesia by using intramuscular injection of 17.5 mg/kg B. Wt. Xylazine and 25 mg/kg B. Wt. Ketamine HCl. In the Ketorolac group, animals were treated by using 30 mg/kg B. Wt. I/M of Ketorolac directly post-operation for 5 days, while control group left without treatment. During the period of the experiments the clinical evaluation directly after operation were recorded in addition to histopathological study of bone defect healing after (7, 14, 28 and 42) days post-operation. The results of this study revealed non-significant changes in redness and heat between two groups; while histopathological findings concerning the activated osteoblasts were noticed in the early stages of bone repair started from day 14 in both groups and continue at all stages of healing, while the woven bone was noticed at 14 days as thin type. Ossification phase was started at 28 days as soft callus and become as hard callus completely at 42 days until it became lamellar bone in both groups these results insure that the two groups recorded no obvious delay effect on healing process. According to the results of this study, treated group could be considered as safe of the NSAIDs drug, which had no harmful effect on experimental animals.

KEY WORDS: Ketoprofen, bone healing, femur, rabbits.

INTRODUCTION

The internationally acknowledged ethic of animal experimentation requires that animal pain and suffering be minimized^[1]. It is surely understood that bone deformities and affection are agonizing, so pain medication is typically a fundamental piece of treatment^[2]. Most general anesthetics have a pain relieving activity, however part from this analgesia can be given by utilizing different agents like NSAIDs; Because of their pain relieving and calming impacts, non-steroidal anti inflammatory drugs (NSAIDs) are among the frequently public prescribed medication^[3]. NSAIDs have possibly deleterious effects on bone metabolism and combinations; several examiners demonstrated that admin analgesic agents post operatively could influence bone-healing processes^[4,5]. The aim of this study was to evaluate the effects of analgesic agents post-operatively on bone healing process based on clinical and histopathological study of femoral bone defect healing.

MATERIALS & METHODS

A total number of 40 adult local breed male rabbits were used in this study and the mean weight was (1.036 ± 0.107) kg. They were divided randomly into two groups in the first (n=20) which represent control group, while in the second group (n=20) which represented treated group (Ketorolac (30mg/kg B.W)). They were housed in the animal house of College of Veterinary Medicine, University Baghdad. They had free accesses to water and food. The animals were left 4 weeks for adaptation with the experimental conditions. Amprolium was used as anti

coccidiosis drug at a dose of 0.6ml/L/18day in drinking water in addition to intramuscular injection of ivermectin at a dose of 0.2 mg/B.Wt rabbit and the dose was repeated after 21days. The animal was fasted for less than six hours and water withdrawn 2 hours and the site of operation was prepared aseptically. Experimental animals were subdued to aseptic surgical conditions and procedures under general anaesthesia by using Xylazine and Ketamine in both groups^[6]. Surgical preparation was all around the operated leg extending from dorsal midline down to the mid-third of the tibia. Then animal was positioned laterally on the surgical table. Longitudinal incision was done at candolateral site of the femur with a line extending from the major trochanter to the lateral condyl of the femur, then the fascia lata was incised separated vastus lateralis cranially and biceps femoris caudally. Bone defect (cavitation) was made in the middle of the femoral diaphysis. The bone was drilled to perform a hole into the all thickness of the cortical parts of the femoral bone 2.3 mm in diameter (figure 1) [7].

Procaine penicillin was used as a local antibiotic to the site of bone defect. The wound was closed by simple continuous suture to approximate the fascia lata with absorbable suture 2/0 pattern and the skin was closed using 2/0, silk, simple interrupted suturing. Samples were taken after 7, 14, 28 and 42 days for both groups (control and treated). Femur bone specimens were taken and kept in formalin buffer solution 1 then washed with distilled water. After washing the autopsies were decalcified by using formic acid and sodium citrate[8] and then serial

routine process to prepare the slices of tissue in which staining by H & E. These slides were examined by light

microscope to observe the difference between the two groups.



FIGURE 1. Showed hole induced in the middle shaft of femoral bone

RESULTS

The results At 7 days post operation in control group showed newly formed granulation tissue in marrow space accompanied by fragment of sequestered dead bone as shown in (Figure 2). Other section showed newly formation of thin spicule of woven bone accompanied with remnant of basophilic ossification primitive. While in treated group there were no obvious differences at 7 days post operation (i.e. well vascular and cellular connective tissue filling the gap of fenestrated area). As well as presence spicules of woven bone in the hemopoietic tissue were seen mainly in periosteum converted in to thin irregular bony trabecular as shown in (Figure 3).

At 14 days post operation in control group, the early newly formed thin woven bone trabeculae was seen at this period mainly in highly vascularized mesenchymal producing connective tissue callus as shown in (figure 4). While other section of microscopical picture showed irregular trabeculae containing remnant of basophilic myloid tissue surrounded with mesenchymal tissues in treated group formation of large areas of woven bone replaced wide space of gap of the fenestrated area separated by cluster of hemopoietic tissue, also the result showed thick bony trabecular filling the gap of the fenestrated area lining with surface osteoblast, between these trabeculae there were few hemopoietic tissue containing cellular infiltration as shown in (Figure 5). Some of bony trabecular were appeared curved with empty lacuna. At 28 days post operation in control group, the microscopical picture

revealed the presence of thick bone trabeculae with active surface osteoblast associated with evidence of resorbed cavities. While other section showed, well-developed lamellar bone containing wide Haversian canal filled the fracture gap as shown in (Figure 6). While in treated group, the majority of lamellar trabeculae were thick, complete lining with active osteoblasts and containing osteocytes in the lacuna. Some of trabeculae were surrounded by small cellular foci of inflammatory cells, while other sections showed evidence of early formation of Haversian canal as shown in (Figure 7).

At 42-days post operation in control group, the predominant feature characterized by cluster of cellular infiltration was seen between bone trabeculae with surface osteoblast lining. Other sections showed that fenestrated area was filled with trabecular bone lining by active osteoblasts and containing calcified center, in addition to compact bone completely filled the artificial space, with osteoclast in the Haversian canal as shown in (Figure 8). In addition to large resorped cavities was observed in compact bone, while in treated group, the lesion was more developed in the later in which the gap of fenestrated area completely replaced by compact bone with marked increase of remodeling lines and wide Haversian canal was seen in other sections as in (Figure 9). Other sections showed elongated branches extended from mature new bone filling the gap of fenestrated area and surrounded by fatty marrow.

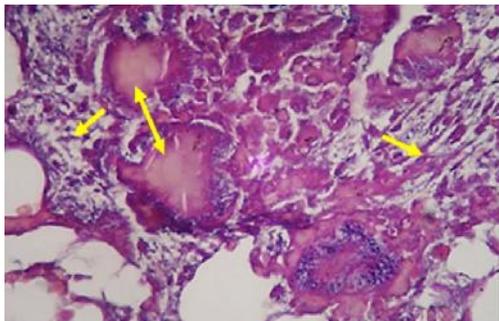


FIGURE 2. Cross section of femoral bone of rabbit after 7 days post operation, In control group. It shows t foci of dead bone sequestra in newly formed granulation tissue (40X H&E stain).

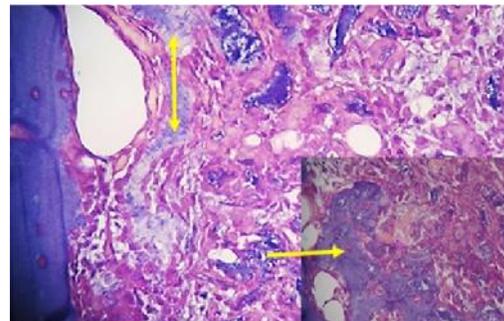


FIGURE 3. Cross section of femoral bone of rabbit after 7 days post operation In treated group; it shows the presence of spicules of woven bone that converted in to thin bone trabeculae deposited in newly bone matrix (40X H&E stain).

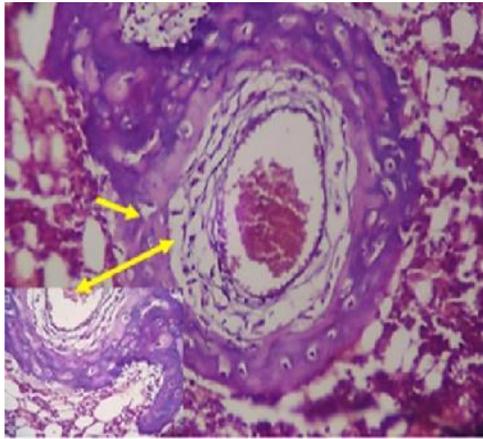


FIGURE 4. Cross section of femoral bone of rabbit after 14 days post operation. In control group. It shows a thin trabecular of woven bone through vascular and cellular connective tissue (20X H&E stain).

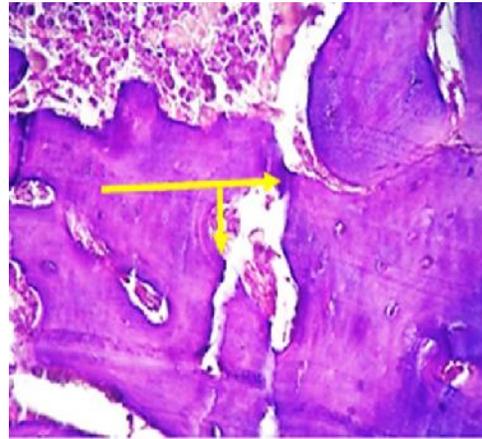


FIGURE 5. Cross section of femoral bone of rabbit after 14 days post operation. In treated group, it shows a thick bone trabeculae with surface osteoblast separated with few hemopoietic tissue (40X H&E stain).

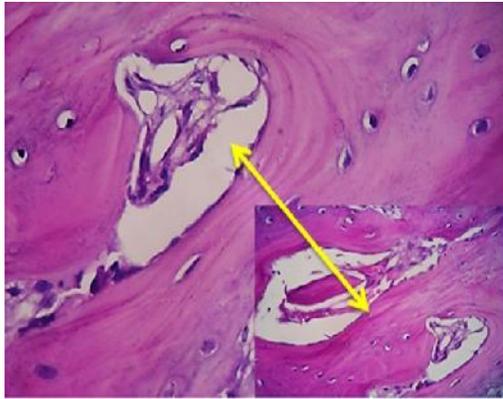


FIGURE 6. Cross section of femoral bone of rabbit after 28 days post operation. In control group. It shows the well-developed lamellar bone containing wide Haversian canal (40X H&E stain).

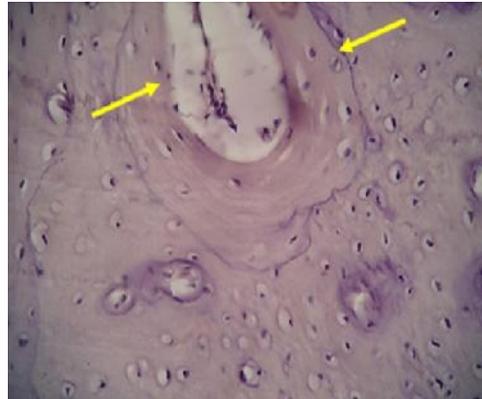


FIGURE 7. Cross section of femoral bone of rabbit after 28 days post operation. In treated group, it shows the early formation of Haversian canal with appearance of attachment line between new & old bone (40X H&E stain).

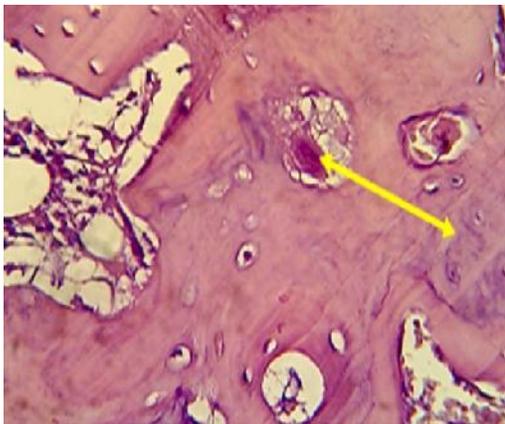


FIGURE 8. Cross section of femoral bone of rabbit after 42 days post operation. In control group. It shows that fenestrated area filled with compact bone with osteoclasts in the HCs (40X H&E stain).

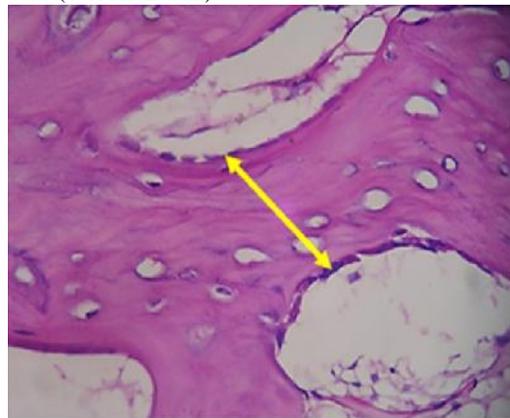


FIGURE 9. Cross section of femoral bone of rabbit after 42 days post operation. In treated group. It shows the wide Haversian canals in compact bone with active surface osteoblast lining (40X H&E stain).

DISCUSSION

Pain is among signs of inflammation, and may be originated from a combination of mechanical factor (break down of collagen) and chemical irritation in addition to neurotransmitters that may generate pain in this condition^[9]. Tissue injury generates several major pain mediators, including but not limited to interleukin-1, bradykinin, K⁺, H⁺, histamine, substance P, and calcitonin gene related peptide (CGRP). Interleukin-1 is an endogenous pyrogen and also upregulates the cyclo-oxygenase gene, leading to synthesis of prostaglandins E₂ and I₂. Bradykinin, K⁺, H⁺, and histamine activate nociceptive afferent nerve fibers and evoke a pain response. Prostaglandins sensitize peripheral nerve endings and facilitate the transmission of painful stimuli along A- and C fibers that reach the cerebral cortex via the spinal cord and the thalamus. Moreover, activated receptors release stored substance P, which itself facilitates the transmission of painful stimuli. Bradykinin, substance P, and CGRP also cause vasodilation and extravasation of fluids that can lead to local swelling and tenderness^[10]. Swelling at the operative area may be due to inflammatory reaction in which the accumulation of the platelets which release proliferation factors like (serotonin, bradykinin, prostaglandins, prostacyclins, thromboxane, and histamines) these factors served in a number of purposes including an increase of cells proliferation and migration to the area and cause blood vessel dilatation, allowing the tissue to become edematous because proteins from blood stream leak into the extra vascular space, which increase its osmolar load and draws water into the area, increased porosity of blood vessels also facilitate the entry of inflammatory cells like leukocytes into the site from the blood stream^[11]. Nonsteroidal anti-inflammatory drugs inhibit Cyclo-oxygenase (COX) 1 and 2. Cyclo-oxygenase inhibitors prevent especially PGE₂ and PGF₂ α induced hyperalgesia. COX inhibitors can control the body temperature and acute/chronic inflammation^[12]. Bone healing is a local process that has an effect on systemic homeostasis. The latter involves vitamins, hormones, enzymes and other factors^[13]. The destruction of the edges of fenestrated area and the presence of fibrin network, inflammatory cells and lysis of RBCs which start from the first day post bone cavitation were in agreement with^[14], they found evidence of increased cell division only 8 hours after injury of the periosteal tissue that developed from the site of the injury. Bone healing following a traumatic or surgical injury is initiated in response to regulatory factors associated with inflammation and the innate immune response. Descriptive studies examining the expression of various peptide-signaling molecules, inflammatory cytokines, and other biochemical mediators of inflammation and repair have shown that prostaglandins are critical to this process and are induced within the first three days after a fracture^[15]. Prostaglandins control osteoblastic and osteoclastic function under physiological or pathological conditions and are important modulators of the bone healing process.

It is well known that osteocytes effects can be noticed via decreasing alkaline phosphatase (ALP), this effect depend on the type of cytokines secreted by this cell which are mainly Interleukin-1 (IL-1) and tumor necrosis factor

(TNF), while osteoclast effect can be noticed via increasing bone resorption and collagenous and these effects depend mainly on IL-1 and TNF also. These cytokines regulate not only immune and inflammatory responses but also wound healing, hematopoiesis, angiogenesis and many other biologic processes^[16].

In our study, Ketorolac showed no adverse effect but according to Cappello^[17] ketorolac has been better studied both in the adult clinical and basic science realms; however, in adult rat and rabbit studies, ketorolac administration has been shown to delay bone healing in some studies but not in others. In adult clinical they, demonstrated increased nonunion rates in patients undergoing posterior spinal fusion who used ketorolac postoperatively.

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