

The effect of tramadole hcl and paracetamol on fracture healing in rat tibia model

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Abstract

In our research, we aim to study the effects of combined usage of tramadole HCl + paracetamol on rat tibia fracture model. In our study, 60 Wistar-Albino type male rats, weighted at 300-350 g., were divided as "control" and "test" (tramadole HCl + paracetamol) groups Under general anesthesia, standard closed fractures were created on right tibias of all rats using blunt ended needle holder with three point principle and then closed reduction and fixing with intramedullary nail (0.7 mm) were provided. After fracture treatment of 30 rats in the test group, starting at the same day, 40mg/kg/day tramadole HCL was introduced as daily intermuscular dosage until they were sacrificed. Rats were sacrificed after the fractures as groups of 10 individuals at 2nd, 4th and 6th weeks. Healing fractured tibias were examined mechanically, radiologically, histopathologically. Results: No positive or negative radiological, biochemical and histological effect was detected after the long duration of tramadole HCL + paracetamol usage, starting from the first day and reaching to 6 weeks, in healing process of closed tibia fractures created and fixed with IM nails in rats. Considering the negative effects of NSAIDs on fracture healing, we think that tramadole HCl + paracetamol combination is a safe option in postoperative pain treatment after fracture.

Keywords: Tramadole HCl, paracetamol, fracture healing

Introduction

Fracture healing is a subject on which continuous researches are carried out. Increasing number of accidents in developing world, growing population of elderly, usage of variety of implants with different qualities, new drugs and their effects cause that the researchers get interested in this subject [1]. Although there is progress in the research of different effects of various factors on fracture healing, and fracture healing subject, still some bone union problems can be seen in some fractures [2]. Primary goals in orthopedic clinics are bringing the patient in a painless state after necessary surgical or conservative interventions at once, and providing patient's mobility as fast as possible by aiding in the fracture healing [3]. Postoperative analgesia is crucial after orthopedic surgery and there are articles on several medicine, used for that purpose, that they retard fracture healing [4]. Tramadole HCl + paracetamol are used in orthopedic clinics separately or together (Zaldiar 20 tbl/Abdi İbrahim) to treat postoperative pain [5,6]. Running across to studies on investigation of tramadole HCl + paracetamol effects on fracture healing during our research lead us to search on this subject.

Materials and Methods

Necessary permissions were obtained from university's Ethics Committee (Date of the decision: 12.24.2009, Session no:2009/9, Decision no:3). Study was carried out in experimental research laboratory. In this study 60 Wistar-Albino type male rats were used. The average age and weight of the rats used in this study was 3,1 months (2.9-3.4 months) and 320 grams (300-350 grams) respectively. Animals were divided into two randomly as control and test groups, then these groups were divided into three in themselves. They were preoperatively monitored for 48 hours in laboratory environment as 10 animals per cage separated into 6 groups in total. Water and rodent feed were provided throughout the study. Animals were monitored at 22 °C, given 12 hours of light and 12 hours of darkness.

For surgical intervention anesthesia, 50 mg/kg Ketamine hydrochloride (Ketalar flakon, Parke Davis, Istanbul) was administered into periton. Anesthesia stat was followed by reaction of the rat to squeezing its skin in every 5 minutes. Rats were covered with green cloth after clean up using Betadine solution.

Skin was penetrated through to right tibia proximal end from tibia plateau anterior face by 0.5 cm anteromedially longitudinal insicion Tibia medulla was opened by entering with 0.7 mm.needle (21 gauge) and tibia fracture was created by pulling the needle back according to bending

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three point principle, and then fixation was provided by placing 0.7 mm injector needle as antegrad intramedullary (Figure 1).



Figure 1. After closed fractures were created on tibia plateau anterior face in accordance with bending three points principle, sending intramedullary to distal fracture fragment by pushing forward the green injector needle with 0.7 mm (21 gauge).

The outer part of the wire inside the canal was cut. The incision point was washed with physiological saline

solution and stitched using primary basic suturing technique with 2/0 silk. Afterwards, the operation was terminated after wiping the wound site with providon iodine solution. The created fracture following the operation on all subjects were confirmed radiologically by direct radiography. Right tibia fracture and intramedullary fixing were verified in all rats. The rats with segmental and open fracture were left out of the study. Antibiotic prophylaxis was not applied during or after the surgical interventions.

Randomly generated six cages used. (Table-1). 50Mg/kg/day paracetamol (Perfalgan 10 mg/ml, 100 ml flakon, Bristol Myers Squibb) [6] and 40mg/kg/day tramadole HCl (Contramal ampul 100 mg/2 mL, Abdi İbrahim) were administered to groups B,D and F intramuscularly. Injections were performed always with insulin needle into left inguinal regions and always by the same person. The rats were killed at 2nd week in groups A and B, at 4th week in groups C and D, and at 6th week in groups E and F. Cervical dislocation technique was used as euthanasia method. The experiment was terminated by sacrificing the rats in medicated groups and control groups by cervical dislocation at 2nd, 4th and 6th weeks in order. No osteomyelitis or death was observed among test subjects in the study. Thus, none of the rat's tibia was not left out of the research.

Table 1. Distribution of groups in tibial fracture created rats

	Group name	Number of rats	Termination
Group-1 Control Fracture + IM fixing	A	10	2nd week
	C	10	4th week
	E	10	6th week
Group-2 Test Fracture + IM fixing + medicine	B	10	2nd week
	D	10	4th week
	F	10	6th week

Right tibias of the sacrificed rats were disarticulated at the knee joints. Soft tissue on the tibia was stripped from the bone without damaging the callus by the pathology specialist, in accordance with histopathological procedures. All of the right tibias were examined as radiologically, histopathologically and biomechanically.

For radiological assessment, direct graphs of control and test groups were obtained by Siemens branded radiography device at 105 cm and magnified to 100%. 10 rat legs were placed according to their groups, on the surface to take graphs at front and the back, and the graphs were taken on a single tape for each group (Figure 2-3).

Samples from the fractured region were collected for histopathological examinations. Collected bone tissue samples were fixated in 10% neutral formaldehyde and kept in 5% formic acid solution. The materials taken into paraffin-embedded blocks after routine histopathological preparation were sectioned into 5mm slices with the aid of Leica Rotary microtome. Obtained sections were dyed with Hematoxylon-Eosin and Hematoxylon van Giesson stains

and examined. Tissue micrographs were evaluated by pathology specialist with digital camera combined binocular research microscope (Figure 4).



Figure 2. Radiography of control and test groups at 4th week



Figure 3. Radiography of control and test groups at 6th week

All slides were assessed due fibrous tissue, cartilage, new bone and mature bone ratios according to the scale recommended by Huo et al. (Table-5,6) [7].

Radiographic and histopathological scores of control and experimental groups were compared (Table-2). In our study 2nd week control and test groups were not assessed as no callus formation was observed radiologically. The graphs of fourth and sixth week control and test groups were evaluated according to the scale, recommended by Lane-Sandhu et al., by two separate observer and compared statistically (Table-3) [8].

Rat tibias were kept in 10% neutral formaldehyde until biomechanical evaluation. Fine wires, sent for intramedullar fixation, were removed. In order to apply bending three point test, all rat tibias in fourth and sixth groups placed in “The TA-XT2i Texture Analyzer“ (Stable Micro Systems Ltd. Godalming, Surrey, UK) which works extension controlled and can transmit the force, applied by a movement with the velocity of 2mm/second, graphically and quantitatively to the computer screen, while in second week control and test groups, because tibia fracture lines were not separated, only 5 of rat tibias of each were mechanically tested.

The resistance of elements in each groups were measured in Newtons applying force to callus region and compared. The results of control and test groups were statistically compared using Mann-Whitney U test (Table-4,6).

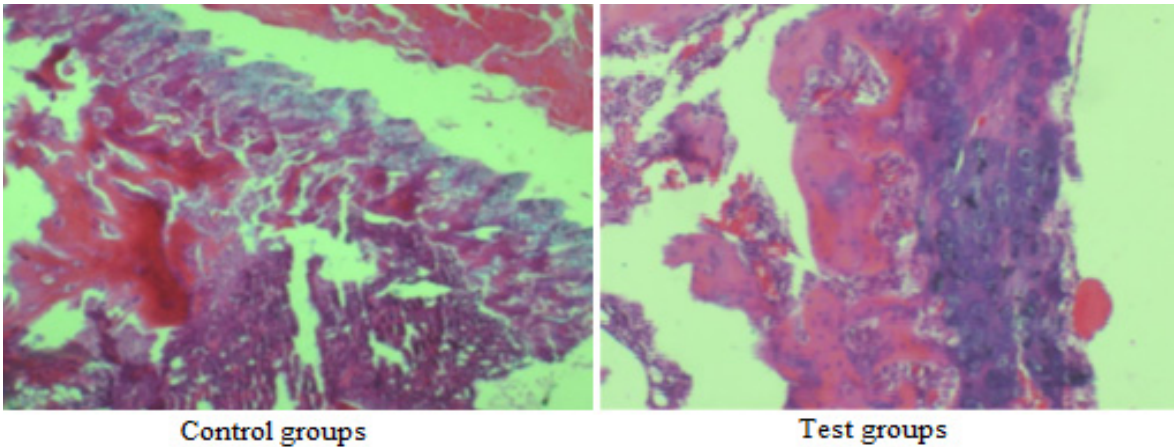


Figure 4. Histopathologic sections: Beginning of fibrous and cartilage tissue formation is observed in second week control and test groups (x100, HTXE).

Table 2. Graph of radiological values in test and control groups

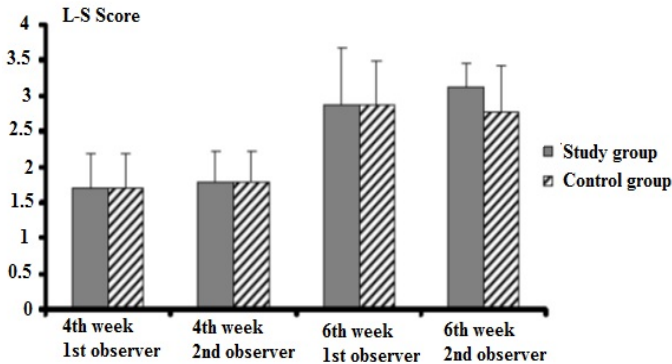


Table 4. Graph of biomechanical values in test and control groups

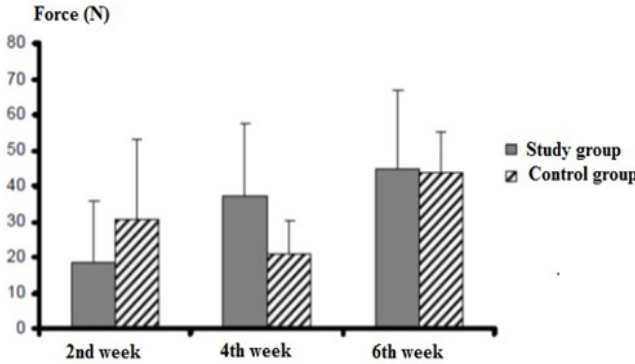
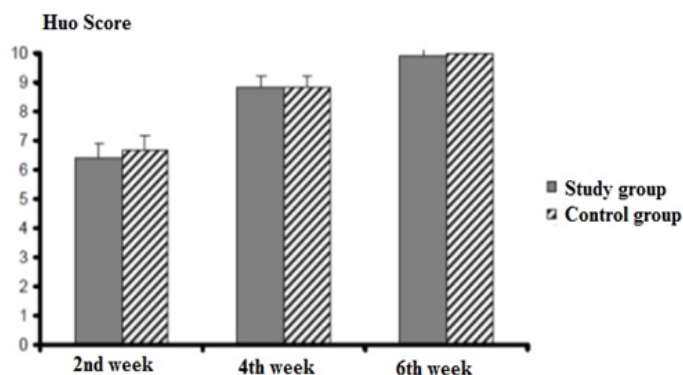
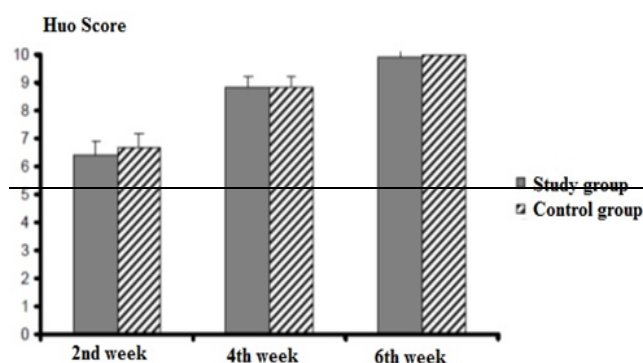


Table 3. Table of radiological values in test and control groups

Groups (n=10)	4 th week 1 st observer		4 th week 2 nd observer		6 th week 1 st observer		6 th week 2 nd observer	
	Study	Control	Study	Control	Study	Control	Study	Control
Radiological scores	1.70±0.48	1.70±0.48	1.80±0.42	1.80±0.42	2.88±0.78	2.88±0.60	3.11±0.33	2.77±0.66
P	>0.05		>0.05		>0.05		>0.05	

Table 5. Graph of histopathological values in test and control groups**Table 6.** Histopathological and biomechanical data:

Results

Second week groups were excluded from evaluation as no radiological union was observed. The differences between average radiological examination values of fourth and 6th week control and test groups were not statistically meaningful ($p>0.05$). The differences between average histopathological examination values of second, 4th and 6th week test and study groups were not statistically meaningful ($p>0.05$). No biomechanical assessment could be done on the rat tibias in second week test and study groups. The differences between average biomechanical examination values of fourth and 6th week test and study groups were not statistically meaningful ($p>0.05$).

Discussion

Relieving the pain of the postoperative patient after fracture treatment is important with regard to life quality [9-11].

The possible positive or negative effects of the agents, that we use in pain treatment, on bone healing are of importance.

There are many medicine used in pain management [12,13]. Postop pain treatment consists typically of opioids, NSAIDs and COX-2 selectively. It has been demonstrated that tramadole is effective on nociceptive and neuropathic pains with its double-acting mechanism (μ receptor agonist and norepinephrine/serotonin reuptake inhibitor).

NSAIDs are noteworthy medicines chosen for postoperative pain treatment. In several studies it has been showed that COX-2 activity is essential for bone healing and stated that COX-2 inhibitors may retard bone healing accordingly [14,15]. In the 8 weeks study by Simon et al. with 253 rats, retarded bone healing was reported in t-rats treated by COX-2 inhibitors 2 days prior to creation of fractures. In a study of 377 patients treated by femoral shaft fracture intramedullar nail by Giannoudis et al., it has been established that high dosage of NSAIDs are responsible for retardation of healing in humans [13].

Alien et al. observed healing retardation in their study with aspirin and indomethacin [14]. Elves et al, in their 8 weeks study, determined a negative effect on rats the medication was started 1 week prior to creation of the fractures in pelvis and vertebra [15]. In the study of Tornkvist et al. with rabbits, they established that in the groups, for which indomethacin and ibuprofen were used, torsional strength did not return to normal in 5-8 weeks unlike the control group [16]. On the other hand, More et al. used proxicam and flunixin on rabbits for 3 weeks and stated that NSAIDs might have retarded but didn't break the healing process [17]. They found that naproxen slows down bone formation in only very high doses and slows down bone resorption in low doses in a study with rats.

Fracon et al. demonstrated that usage of ketorolac, etoricoxib and paracetamol in rats has no negative effect on alveolar bone healing after 2 weeks treatment [18].

In literature there was no study on the effect of tramadole HCl + paracetamol combination (Zaldiar 20 tbl/Abdi İbrahim) on fracture healing [19,20]. This medicine is frequently used in surgical clinics to kill the after fracture and postoperative pain for its strong analgesic effect and reduced gastrointestinal side effects.

Paracetamol and tramadole HCl were administered separately as the animal dosages of paracetamol and tramadole in literature search were not consistent with the doses present in medicine combination [21].

In our study 2nd week control and test groups were not assessed as no callus formation was observed radiologically. No statistically meaningful difference was

seen between fourth and 6th weeks test and control groups' images.

In the biomechanical evaluation there were similarities between 2nd, 4th and 6th weeks. The average of fracturing force was increasing day by day and this showed that the strength of the callus tissue was improving.

Samples were collected at proximal and distal regions during histopathological evaluations. We think that it is a better way for evaluation. In second week histopathological examination, union origin was present and the microscopic images were alike. This demonstrates that our medicine has no positive or negative effect on fracture healing in the short term. In statistical study, results of 2 groups demonstrated that. We observed that callus tissue is larger and microscopic images are alike in examinations for fourth and 6th weeks.

These findings proved that our medicine does not affect fracture healing. Radiological, biomechanical and histopathological results made us decide that tramadole HCl + paracetamol combination does have no effect on fracture healing. We believe that the validity of our results will increase with larger number of rats and more than one pathologist.

As a result, long duration of tramadole HCl + paracetamol usage on rats starting from the first day and reaching to 6 weeks, has no effect on healing process of closed tibia fractures created and fixed with intramedullary nails in rats. Considering the negative effects of NSAIDs on

fracture healing, we think that tramadole HCl + paracetamol combination is a safe option in postoperative pain treatment after fracture.

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