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THE EFFECT OF 2 AND 14 DAY TREATMENT WITH ASPIRIN, DICLOFENAC AND THEIR COMBINATION ON FRACTURE HEALING IN RABBITS

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ABSTRACT

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) continues to expand at a remarkable rate due both to their broad spectrum clinical applications. NSAIDs are particularly important to patients with a variety of musculoskeletal conditions and injuries. With mounting evidence that NSAIDs do in fact interfere with proper bone healing in various animal models, questions have arisen regarding the differences between the short-and long-term treatments with these drugs on fracture healing, in addition to, the effect of their combined treatment.

Left tibias of 42 male rabbits were fractured by manual pressure under general anaesthesia and are stabilised by Zimmer plate. These animals are divided into seven groups; 6 rabbits each: group (1), control group, given normal saline; group (2), given aspirin (25mg/kg/day) for 2 days post-fracture; group (3), given aspirin for 14 days post-fracture; group (4), given diclofenac (2.5mg/kg/day) for 2 days post-fracture; group (5), given diclofenac for 14 days post-fracture; group (6), given aspirin and diclofenac for 2 days post-fracture; and group (7), given aspirin and diclofenac for 14 days post-fracture. Drugs were administered intramuscularly.

All animals were sacrified 21 days after fracture. At this time, evaluation of fracture healing was performed blindly by two radiologists and a histologist according to a 5- point scale of a descriptive assessment and scores of each group were compared with control and other groups. Both aspirin and diclofenac, when given indivdually for 14 days, produced a statistically significant inhibitory effect on fracture healing, but they have no significant effect when given for 2 days. Administration of aspirin and diclofenac together had a significant delaying effect on fracture healing even after short duration (2 days). The latter effect appeared mainly in histological examinatiom indicating that histological methods of assessment may be more valuable than radiological ones.

It is, therefore, concluded that aspirin and diclofenac should cautiously be used during fracture healing; firstly, they should be given for the shortest possible duration and secondly, should not be given in combination.

Introduction

Fracture healing is a physiological process by which bone regenerates itself following injury[1]. This process occurs through five stages. These are stage of haema-toma,inflammation and cellular proliferation, callus formation, conso-lidation and remodeling[2]. They are not

sharply demarcated and that two or more stages may be seen at the same time in different parts of the bone[3].

This process is affected by a variety of factors including biological, mechanical, biophysical and pharmacological factors[4-8]. An important pharmacological group affecting fracture healing is non-steroidal anti-inflammatory drugs (NSAIDs), which act mainly by inhibiting prostaglandin synthesis. Prostaglandins (PGs) have been shown to elicit and participate in inflammatory responses, increase osteoclast activity and subsequent bone resorption, and increase osteoblast activity and new bone formation[9-12]. In addition to their role in the inflammatory cascade, PGs appear to have a direct effect on the process of bone resorption[13] and served as powerful stimulators of this resorption[12]. It has been shown that PGs could directly enhance bone formation by increasing the replication and differentiation of osteoblasts[14,15]. This apparent integral role for prostaglandins in the process of bone healing, coupled with the knowledge that NSAIDs act by inhibiting the production of prosta-glandins, result in an understanding of the likely mechanism through which NSAIDs may impair bone healing. Different types of NSAIDs have been investigated in experimental fractures and found to have inhibitory effects. These include; diclofenac[16,17], aspirin[18] indomethacin[18-23], ketorolac[24], COX-2 inhibitors[25-26], ibuprofen[20,28], naproxen[25,29] tenoxicam[30] and phenylbutazone[31]. The duration of drug treatment in most of these studies is more than one week. In addition, there is only one study concerning the effect of aspirin on fracture healing, where it was inhibitory only at the highest dose level[18], and no study on the effect of NSAIDs if given in combination. Therefore, the aim of this study is to investigate the effect of diclofenac and aspirin given singly or in combination for short (2 days) and longer (14 days) duration of treatment.

Materials and Methods

Forty two young (2-3 months old) male rabbits, weighing 1 to 1.25 Kg, were used. They were housed under controlled conditions of temperature and lighting. Same diet was given for all animals. Rabbits were left for acclimatization for at least one week before experiment. All rabbits were generally anaesthetised by intravenous injection of ketamine (10 mg/kg) through external marginal auricular vein. Left tibias of all anaesthetised animals were fractured manually. Fixation of fractured bone was made by zimmer plate (a plate used mostly in splinting of finger fractures in human). Animals were divided into seven groups as shown in (table I). The drugs used are; Acetylsalicylic acid (Draspir. Greece, equivalent to 500 mg of lysine acetylsalicylic acid vial), Diclofenac sodium (Clofen, Julphar, 75mg/3ml ampoule), and normal saline for the control group. The animals were subjected to two intramuscular(gluteal) injections daily, one on each side for 14 days.

Two methods were used for evaluating the process of bone healing. These are radiological and histological examination. Radiological evaluation of fracture healing was made blindly 21 days post-fracture. Evaluation was performed according to a 5-point scale describing the degree of callus density (degree of mineralization) and of fracture line visibility.

All animals had been sacrificed 21 days after fracture, and the fractured bones were sent for histological assessment of the healing process

which was performed and examined blindly by a histopathologist. The scoring system consists of 5 points depending on the presence and amount of cellular tissue, woven bone and lamellar bone[1,2]. Bone specimens were decalcified using 10%HCl and washed for 24 hours in running tap water. Sections were processed and paraffin embedded and the slides were stained by hematoxin and eosin[31].

Statistical analysis of data was made using non-parametric tests for radiological and histological scores. Mann-Whitney *U* test was used to test significance. *P* value less than 0.05 was taken as statistically significant[32].

Group	Drug intra-muscular injection(daily)	Duration
1	Nomal saline (0.25 ml)on each side as control	14 days
2	Diclofenac sodium (2.5mg/kg) Nomal saline (0.25 ml)	2 days
3	Aspirin (25 mg/kg) Nomal saline (0.25 ml)	2 days
4	Aspirin (25mg/kg) Diclofenac sodium (2.5mg/kg)	2 days
5	Diclofenac sodium (2.5 mg/kg) Nomal saline (0.25 ml)	14 days
6	Aspirin (25mg/kg) Nomal saline (0.25 ml)	14 days
7	Aspirin (25mg/kg) Diclofenac sodium (2.5mg/kg)	14 days

Table I: Types, doses, and duration of drugs used in the our study

Results

Effect of diclofenac on fracture healing

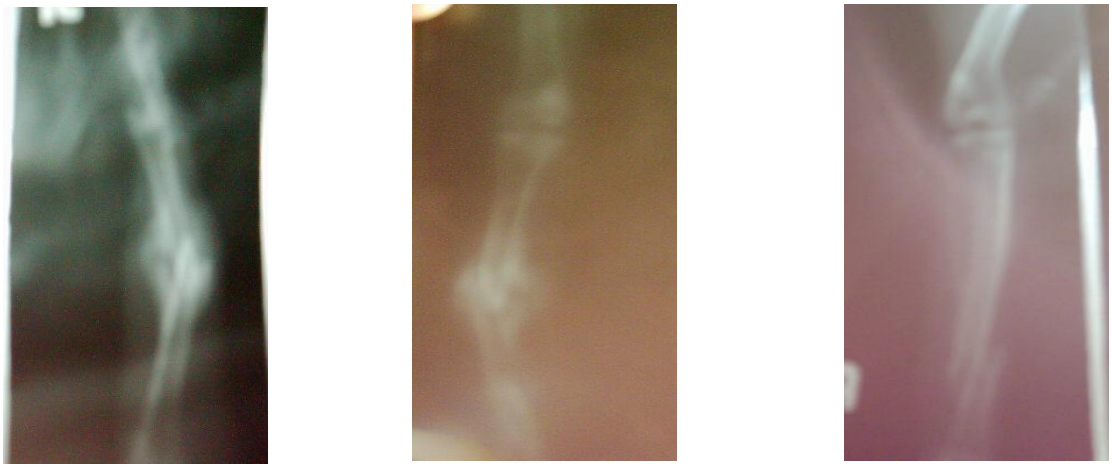
Administration for 2 days: Diclofenac (2.5mg/kg/day) given for 2 days resulted in only 7% and 5% delay in fracture healing in radiological and histological scores in comparison with control respectively; This delay is not statistically significant(table II).

No.	Group	Radiological score		Histological score	
		Mean ± SD	% Reduction	Mean ± SD	% Reduction
1	Control (normal saline)	2.50 ± 0.5		3.67 ± 0.52	
2	Aspirin for 2 days	2.17 ± 0.75	13%	3.50 ± 0.54	5%
3	Diclofenac for 2 days	2.33 ± 0.52	7%	3.50 ± 0.54	5%
4	(Aspirin + Diclofenac) for 2 days	2.17 ± 0.75	13%	2.83 ± 0.54	23%*
5	Aspirin for 14 days	1.66 ± 0.82	33%*	2.50 ± 0.55	32%*
6	Diclofenac for 14 days	1.50 ± 0.84	40%*	2.17 ± 0.52	41%**
7	(Aspirin + Diclofenac) for 14 days	1.50 ± 0.55	40%*	2.00 ± 0.63	46%**

Table II: Effects of aspirin and diclofenac treatment on fracture healing of rabbit tibia when assessed 21 days after fracture presented as radiological and histological scores. Significant differences with respect to control: * $P< 0.05$, ** $P< 0.01$

In radiological examination (21 days after fracture), there was no clear difference in the density of callus formation between control group and that treated with diclofenac for 2 days after fracture (figure 1).

In histological examination (21 days after fracture), there was a good amount of woven bone with a remnant of cellular tissue; a picture comparable with control group.



(a) (b) (c)

Figure 1: X-ray films showing the effect of diclofenac on fracture healing 21 days after facturing rabbit tibia in comparison with control.(a) Control; good callus formation, (b) Diclofenac for 2 days; clear callus appearance, (c) Diclofenac for 14 day; poor callus formation.

Administration for 14 days There was a statistically significant delay in fracture healing in those treated with diclofenac for 14 days when assessed radiologically (40% reduction, $P < 0.05$) and histologically (41% reduction, $P < 0.01$) in comparison with control group (table 2) .

Radiological examination 21-day post-fracture showed that the density of callus formation in animals treated with diclofenac for 14 days is much reduced compared with control group (figure 1). Histological examination also showed a delay in the healing process where the amount of woven bone is scanty in comparison with cellular tissue 21 days after fracture.

Effect of aspirin on fracture healing

Administration for 2 days. There is no statistically significant difference between control group and that treated with aspirin for 2 days in both radiological and histological scores (table II).

There was a comparable density of callus formation in the control group and the group given aspirin for 2 days when examined radiologically 21 days after fracturing the tibia. Histological examination of the fracture site at same time, also revealed no difference between these two groups.

Administration for 14 days. There was a statistically significant reduction in radiological score (33%, $P <0.05$) and histological score (32% , $P < 0.05$) caused by aspirin given for 14 days with respect to control (table II). Aspirin given for 14 days post-fracture resulted in a clear declining of the density of callus formation with respect to control. Histologically, the amount of cellular tissue is greater than that of woven bone when compared with control which indicates that aspirin given for 14 days caused a delay in bone repair.

Effect of the combination of diclofenac and aspirin on fracture healing

Administration for 2 days

There was only 13% reduction in radiological scores of animals injected with the combination of aspirin and diclofenac for 2 days in respect to control; this reduction is not statistically significant. However, in histological score, there was a statistically significant reduction (23%, $P < 0.05$) compared with control (table II).

There was a good density of callus formation in radiological examination 21 days after fracturing the tibia in those animals receiving both aspirin and diclofenac for 2 days, which is comparable with that of control animals (figure 2).

In histological examination, the development of woven bone at fracture site on 21-day post-fracture is reduced in animals treated with combined administration of aspirin and diclofenac as compared with control group.

Administration for 14 days. The scores of radiological assessment are reduced by 40% with respect to control when combined aspirin and diclofenac were given for 14 days post-fracture; and this reduction is statistically significant ($P<0.05$). In addition, the reduction in the mean of histological scores was 46% in comparison with the control group, which is also statistically significant ($P < 0.01$) (table II, figure 2).

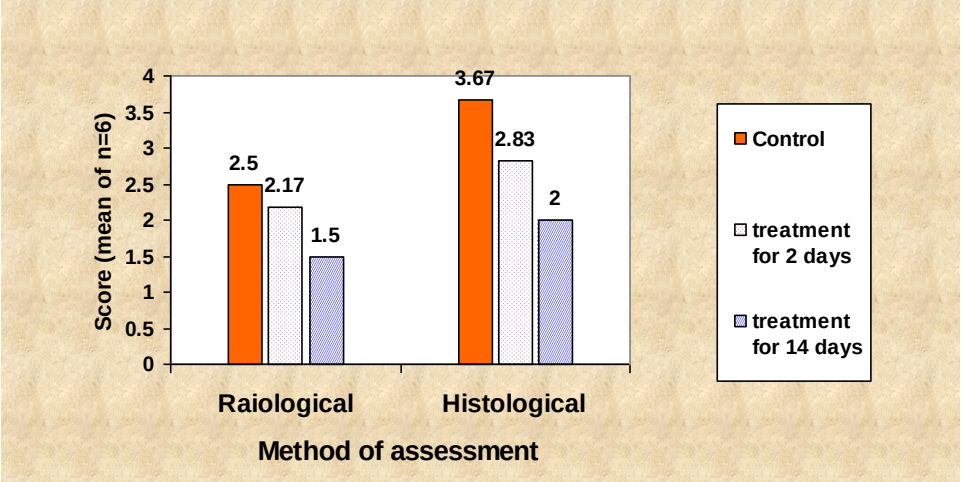


Figure 2. Effect of combined administration of aspirin (25mg /kg/day) and diclofenac (2.5mg /kg / day) on fracture healing of rabbit tibia assessed radiologically and histologically 21 days postfracture in comparison with control.

Comparison between single and combined treatments on fracture healing

In radiological examination**,** there was no significant difference in bone healing between animals treated with combined aspirin and diclofenac and those treated with aspirin or diclofenac alone for 2 days post-fracture (figure 3a), but the difference between the above cited groups in histological examination is statistically significant (*P<0.05*) (figure 3b). On the other hand, both radiological and histological scores indicated that there is no significant differences between these groups when drugs given 14 days after fracture.

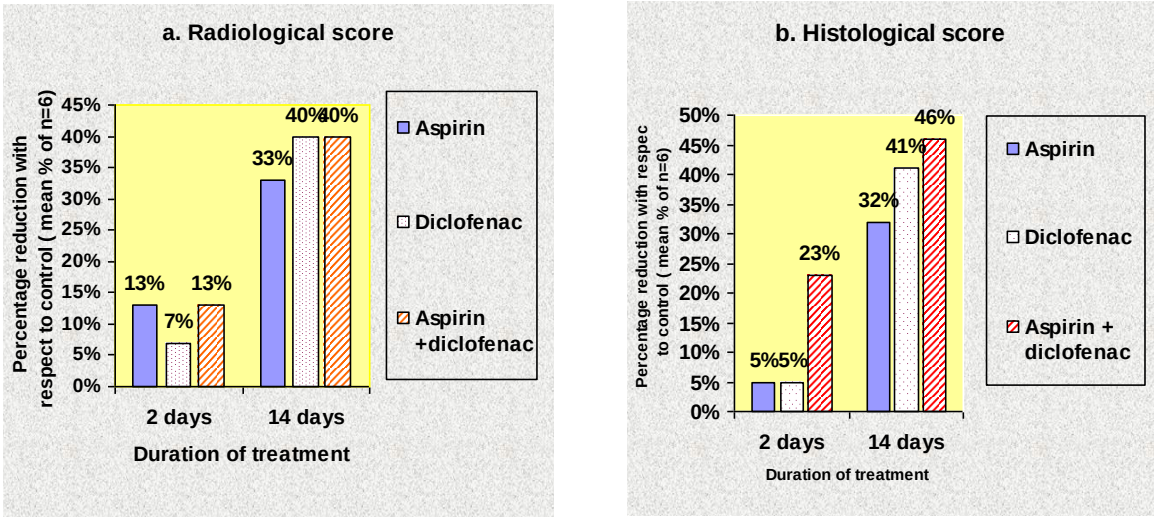


Figure 3: Comparison between the effect of combined aspirin (25mg/kg/day) and diclofenac (2.5mg/kg/day) and that of each of these drugs alone on fracture healing of rabbit tibia assessed radiologically and histologically 21 days postfracture.

Discussion

NSAIDs are widely used as analgesics in patients with fractures, including paediatric patients, those with stress fracture and adult traumatic fractures[33]. Unlike other tissues that heal through the generation of scar tissue, bone heals by regenerating new bone. When a fracture or some other bone trauma initially occurs, it disrupts the local blood supply to the bone, ultimately causing death of the cells in that area. An aseptic inflammatory response results, followed by the resorption of necrotic tissue and the proliferation and differentiation of pluripotent osteoprogenitor cells leading to the production of new skeletal matrices[34] Thus, an inflammatory response, bone resorption, and bone production are required for proper bone healing.

At sites of injury and inflammation, macrophages, fibroblasts, and synovial cells release COX-2, which subsequently upregulates the production of PGs involved in the inflammatory response[35]. Prostaglandin production and COX-2 mRNA are increased in fracture callus during the first two weeks following injury, suggesting a role for these agents in the early phase of bone healing[24].

A body of evidence is now available for the delaying effect of NSAIDs on fracture healing. On the other hand, few studies reported different effects of some NSAIDs on fracture healing. A study by Akman et al[38] in 2002 revealed no effect of diclofenac on fracture healing in rats when given for 10 days post-fracture, where despite subjective inhibition of fracture healing by clinical examination, radiological evaluation was not significant at the end of 2 weeks. At 4 and 6 weeks post-fracture, clinical, radiological and histological findings were comparable among groups[36.] In our study, radiological assessment demonstrated that diclofenac had a significant delaying effect on fracture healing of rabbit tibia when given for 14 days post-fracture. This effect appeared as a low density callus formation in x-ray films compared with control group. The delaying effect of diclofenac on fracture healing is more significant in histological assessment as shown by presence of little amount of woven bone compared with control group.

Our findings are in agreement with the results of studies performed by Beck et al[16] and Matziolis et al[17].

The lack of effect in Akman study[36] may be due to the reversibility of the inhibitory effect of diclofenac at 4 weeks post-fracture. Reversibility of the inhibitory effect has been reported for some NSAIDs such as indomethacin[20].

In the present study, aspirin caused, a statistically significant, reduction in callus density in radiological examination when it is given in a dose of 25mg/kg/day for 14 days post-fracture and a delay in woven bone development in the histological examination when compared with control groups.

Although, the analgesic and antipyretic dose of aspirin in human is 25-50mg/kg/day for adult and 50-75mg/kg/day for children, Allen et al[16] in 1980 used very high doses of aspirin (100, 200, or 300 mg/kg/day) in their study on fractured ulnar bone of rats. They stated that the inhibitory effect occurred only with the higher doses. No reasonable explanation was given for using such high doses of aspirin by Allen et al,[18] and no other reports about aspirin effect on fracture healing could be found in the literature.

Although, there was no statistically significant difference between the

effect of aspirin and diclofenac on bone healing, diclofenac seems to have more delaying effect on fracture healing than aspirin.

Up to our knowledge, there is no report on the effect of short-term administration of NSAIDs (i.e less than 7 days after fracture) or the effect of NSAIDs used in combination on fracture healing, which is a common practice in treatment of fractures here, in Basrah. For these reasons we investigated these two aspects in the present study.

The effect of 2-day treatment with aspirin and diclofenac, given individually, on fracture healing of rabbit tibia revealed a slight and insignificant difference compared with control in radiological and histological assessments 21 days post-fracture (reductions in radiological scores by 13% and 6.5% for aspirin and diclofenac drugs respectively, and 5% reduction in histological scores for both drugs). The lack of effect might be due to either there is no real effect for these drugs when they are given for a short time or the inhibitory effect is reversed quickly after stopping the drugs as similarly reported with COX-2 inhibitors[25]. However, administration of both aspirin and diclofenac together for 2 days resulted in a slight and insignificant delay in fracture healing when assessed radiologically (13% reduction with respect to control), but this delay is more clear and statistically significant in histological assessment (23% reduction with respect to control, $P<0.05$). The poor correlation between the radiological and histological findings, is in accord with the results of the study of Blokhuis et al.[37] The latter stated that plain radiography provides poor parameters for monitoring fracture healing process, and there is a poor correlation between radiographic and histological data[37].

Giving the two drugs in combination for 14 days post-fracture resulted in a delay in fracture healing of rabbit tibia in both radiological and histological evaluation 21 days post-fracture. There was no statistically significant difference between the effect of aspirin or diclofenac, given individually for 14 days, on fracture healing and that of their combination. However, the extent of this delaying effect is more evident with combined administration of aspirin and diclofenac as shown in figures.

It can, therefore, be concluded that aspirin and diclofenac caused a significant inhibitory effect on fracture healing of fractured rabbit tibia when given individually for 14 days post-fracture, but they did not have harmful effect if used for 2 days. The delaying effect of combined administration of aspirin and diclofenac for 14 days after fracture is more evident than each of these drugs given alone. In addition, their combined use could be harmful even if used for 2 days after fracture.

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