

*Full Length Research Paper*

# Clinical and histopathologic impacts of indomethacin on healing of experimental gum injuries on rabbits

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Indomethacin is one of the old non-steroidal anti-inflammatory drugs and it inhibits cyclooxygenase I and II. The objective of this research is to study the clinical and histopathological effect of indomethacin which is usually used in surgery as an analgesic drug and which is evaluated on the process of healing of surgical wound. We selected 15 New Zealand white rabbits with the same condition and after numbering, they were divided into three groups of 5 rabbit in each group. Then, in each group after anesthesia with xylazine and ketamine, a surgical wound was created in the right lateral region of mandible with biopsy punch and then, after 12 days, clinical and histopathological effects of drug evaluated in low dose and high dose groups when compared with control group that received placebo. It was evident that the use of this drug had positive effects on healing, and this was also obvious in clinical and histopathologic findings in both low dose and high dose groups when compared with control group. Also use of low dose of indomethacin had better effects on healing process after surgical trauma.

**Key words:** Indomethacin, healing, gum injuries, rabbit.

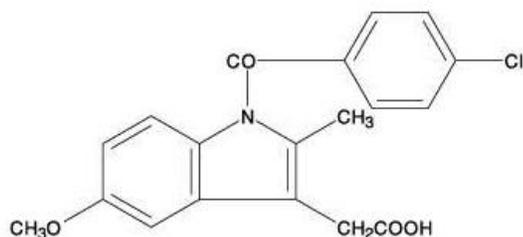
## INTRODUCTION

Indomethacin is a non-steroidal anti-inflammatory indole derivative designated chemically as 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid. Indomethacin is practically insoluble in water and sparingly soluble in alcohol. It has a pKa of 4.5 and is stable in neutral or slightly acidic media and decomposes in strong alkali. The structural formula of indomethacin is as shown in Figure 1.

Non-steroidal anti-inflammatory drugs which is known as NSAIDs inhibits prostaglandin synthesis by the gastric mucosa and thromboxane production by platelets so impairing platelet aggregation. Within 90 min of acute aspirin ingestion in humans, extensive intramucosal petechial hemorrhage occurs visibly and this may, in part, be related to promotion of bleeding from the antiplatelet

actions of aspirin. With longer term of ingestion, the number of erosions may diminish, possibly by a process of adaptation (Graham et al., 1988b). However, erosions, petechiae and superficial ulcers are quite common in patients on long-term maintenance treatment with NSAIDs. Moreover, it is not established why a small proportion of patients develop chronic ulcers yet others exhibit little or no mucosal damage. Among the mechanisms proposed for delayed healing are: A, inhibition of synthesis of prostaglandins that are important for gastroduodenal mucosal defense (Hirose et al., 1991; Graham et al., 1988a); B, inhibition of epithelial cell proliferation in the ulcer margin that is critical for re-epithelialization of the ulcer crater (Inauen et al., 1988; Schmassmann et al., 1994; Levi et al., 1990); C, inhibition of angiogenesis that is essential for nutrient supply in the ulcer bed (Halter et al., 1992) and D, inhibition of proliferation and function of myofibroblasts involved in remodeling and contraction of the granulation tissue in the ulcer bed (Schmassmann et al., 1994; Ogihara

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**Figure 1.** Structural formula of indomethacin.

and Okabe, 1993). Indomethacin is used to relieve pain, swelling and joint stiffness caused by arthritis, gout, bursitis and tendonitis. Reports have appeared in the literature suggesting that indomethacin may be useful in rehabilitation of patients who have suffered fractures or who have undergone orthopedic surgical procedures (Bernstein et al., 1977; Dittmar and el-Salamouny, 1968; Kudo et al., 1968; LeClerc and Autissier, 1969). However, recently some questions have been raised concerning the possibility of delay of fracture healing by indomethacin (Huusko et al., 1975; Ro et al., 1976; Sudmann, 1976; Sudmann and Hagen, 1976).

## MATERIALS AND METHODS

In this study, 15 New Zealand female rabbits weighing 1.5 kg were selected. All animals are 3 months old. All rabbits were developed under laboratory conditions to prevent common infections, drug interactions, etc. These rabbits were numbered by chance with a waterproof marker in the inner layer of ear and were allocated into 3 groups by chance. Group 1: (rabbits from 1 to 5) as control group did not received indomethacin. Group 2: (rabbits from 6 to 10) received low doses of indomethacin. Group 3: (rabbits from 11 to 15) received high doses of indomethacin. Conditions, such as temperature, humanity, bed, light and ventilation were provided, same for all animals. Storage temperature was 25°C.

### Pre-operation measures

The operation (induction wound in the gum) required general anesthesia, analgesia and muscle relaxation. In term, we used ketamine (10%, 35 mg/kg) and rampon (2%, 5 mg/kg) for the induction of anesthesia and pre-operation drugs, respectively.

### Operation measures

To making wound in the rabbits gum in the same size, we used sterile biopsy set and biopsy punch with 6 mm in diameter, samples from right side of gum were approached and samples were fixed in the formalin (10%) and was sent to pathology laboratory.

### Post-operation measures

After operation, first dose of indomethacin was given to treatment group rabbits in with low and high doses, and in the same volume serum physiology was given to the control group rabbits.

Indomethacin was administered in two low and high doses. Group 3 (high dose) received 1 mg/kg indomethacin daily and group 2 (low dose) received 0.5 mg/kg indomethacin daily.

### Final sampling

12 days after feeding of indomethacin to the treatment groups and normal saline to the control group, samples from healed tissues were obtained and sent to laboratory. Sampling from healed tissues also required anesthesia; hereby, we used ketamine and rampon, for the first day. In laboratory, slides were provided and were stained by Hematoxylin and Eosin method (H and E).

### Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean  $\pm$  standard error of the mean (SEM). Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by one-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple comparison test.  $P < 0.05$  was considered statistically significant.

## RESULTS

In clinical view, healing differences from day 5 were seen among control and treatment groups' rabbits. These differences were significantly increased such that on day 13 (final sampling day), significant difference was seen between the control and treatment groups; although the treatment groups had good healing than the control group. The result also revealed that in the low dose group, the healing effect was better than in the high dose group.

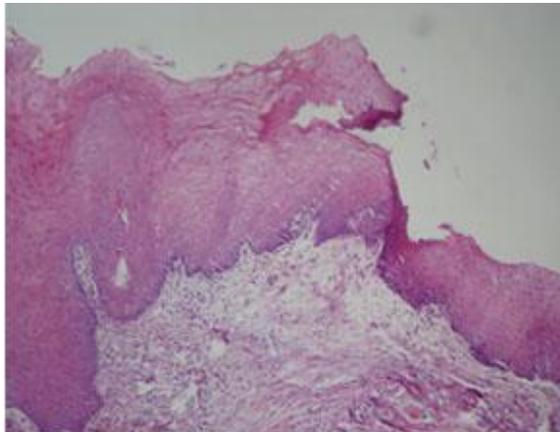
### Pathologic findings

Histopathology revealed that healing in the treatment groups was more than control group. This finding is obviously approved by looking at the epithelial. In low dose group, healing occurred in a higher extent than in the high dose group as it detected by checking the epithelial. In high dose group, healing process was very slow and existence of inflammatory cells and hyperemia in healing area was more intense than in the low dose group. This indicates that high dose of this drug is less effective (Figures 2 to 7) on the healing process and cessation of inflammation.

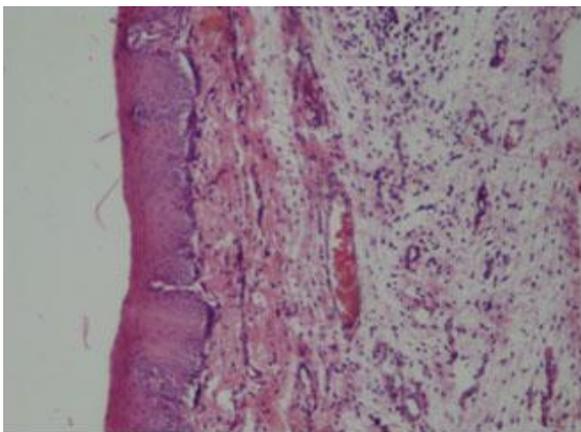
Based on Table 1, all measured factors in low dose treatment are higher than high dose treatment group.

## DISCUSSION

The mechanism of action of non-steroidal anti-inflammatory



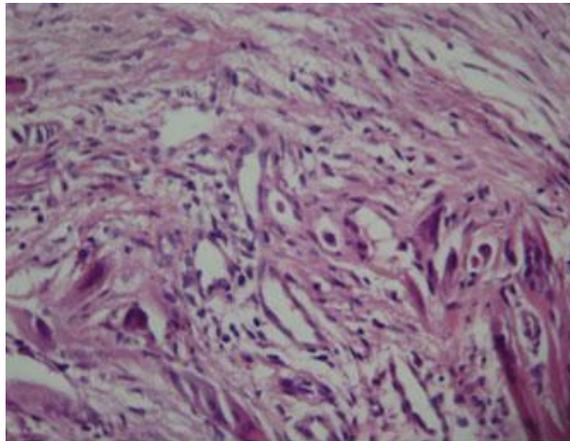
**Figure 2.** Microscopic view of healing process from one rabbit of the low dose treatment group. Epithelial gap because of regeneration of lining tissue has disappeared. Newly formed epithelial is disorganized and irregular, and thicker than the adjacent epithelial tissue. Epithelial cells have degenerative changes as cloddy swelling. New formed granulation tissue is immature and has low density of fibrous strands. Edematous changes in this tissue are obvious and new formed vessels indicate active angiogenesis. (H and E, 40x).



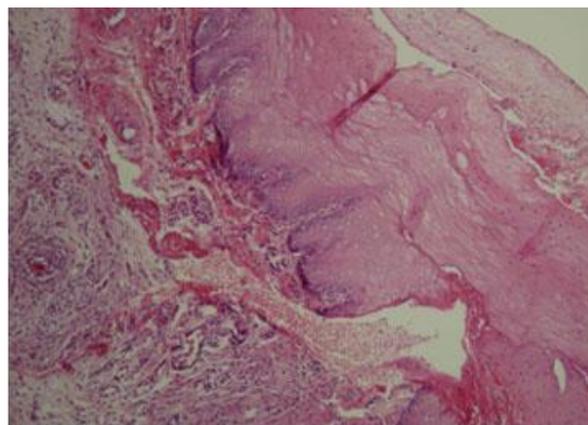
**Figure 3.** Microscopic view of healing process from one rabbit of the low dose treatment group. Numerous new formed vessels with fibroblasts in the location indicate an active healing. Inflammatory cells are existing in the fibrous filaments. Foreign body type of giant cell is obvious that indicates tendency of healing tissue to making epolis. (H and E, 100x).

drugs (NSAID) had only recently been discovered when the drugs' ability to inhibit bone healing was first documented. Ro et al. (1976) reported significantly reduced fracture healing in rats treated with indomethacin.

In the same year, a 64-year-old man with an ankle fracture/luxation was purposefully treated with indomethacin to prevent callus formation in anticipation of surgery (Sudmann and Hagen, 1976). In a study by Allen



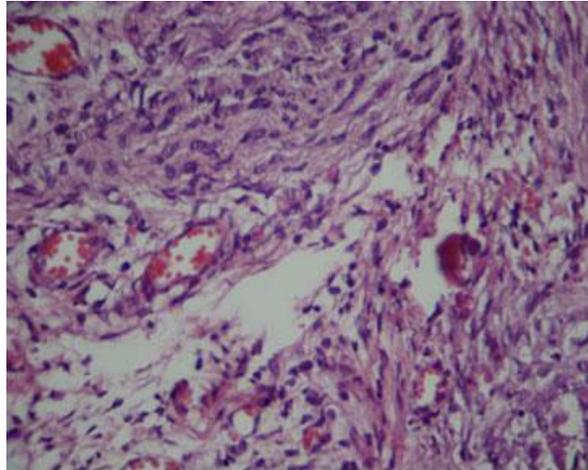
**Figure 4.** Microscopic view of healing process from one rabbit of the high dose treatment group. Epithelial gap because of regeneration of lining tissue has disappeared. Newly formed epithelial are relatively organized and regular. Granulation tissue has more maturity than low dose treatment group and collagen fibers have more regularity and intensity. Thus, edematous changes in profound layers is severe and hyperemia and infiltration of inflammatory cells are more obvious. (H and E, 40x).



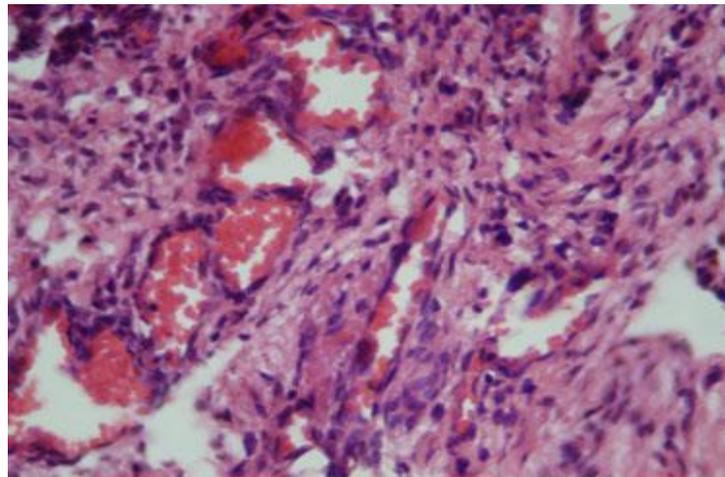
**Figure 5.** Microscopic view of healing process from one rabbit of the high dose treatment group. Hyperemia, inflammation and expansion of the interstitial spaces because of edema were more obvious. (H and E, 100x).

and Wase (1980), they worked on indomethacin and aspirin: effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat revealed that there was a drug and dose-related retardation of fracture healing, which was statistically significant at all dose levels of indomethacin and the highest level of aspirin, as compared to controls (Bo and Sudmann, 1976).

Decreased mean grades in the groups given 100 and 200 mg/kg/day of aspirin, though not statistically significant, suggest a retarding effect on fracture healing at these levels also. No statistically significant changes in the number of pseudoarthroses were found. In one other study



**Figure 6.** Microscopic view of healing process from one rabbit of the control group. Newly formed epithelial tissue did not cover completely epithelial gap and incomplete areas were covered by growth and development of lining tissue from adjacent areas. Existence of fibrin discharges in the superficial lining tissue indicates incomplete healing. Hyperemia, sever hemorrhage and inflammation under the new formed lining tissue is obvious. Newly formed epithelial cells are not organized and regular. (H and E, 40x).



**Figure 7.** Microscopic view of healing process from one rabbit of the control group. Hyperemia and hemorrhage in healing tissues are obvious. (H and E, 100x).

**Table 1.** Comparison of assessed parameters in 3 groups.

Group	Healing parameter					Summary of healing process
	Tissue damage after surgery trauma	Hemorrhage	Fibroplasia process	Inflammation	Regeneration	
Control	3	4	3	2	5	17
Low dose treatment	5	6	5	4	7	27
high dose treatment	4	5	4	3	6	22

by Bertsch and Marks (1982) on comparative study on wound-healing in neonatal and adult mouse epidermis *in vivo*, revealed that the response in adult mouse epidermis is inhibited by local application of indomethacin, whereas the response of the newborn epidermis is not. Sudmann (1976) worked on the effect of indomethacin on fracture healing in rats and demonstrated that the healing of closed, non-immobilized femoral fractures in rats was seriously impaired by indomethacin given orally at a dose of 2 mg/kg daily. The fracture haematomas were larger and disappeared later in the animals receiving indomethacin.

In a study by Carlstedt and Madsen (1986) entitled influence of indomethacin on collagen synthesis during tendon healing in the rabbit, it was shown that indomethacin affected the collagen metabolism differently depending on whether the tendons were involved in wound healing or not. In intact tendons, the drug caused a small general inhibition of collagen synthesis. In the healing tendon, there was a shift towards the synthesis of more insoluble collagen with little effect on the total synthesis. After 4 weeks, there was also a slight but significant decrease in the amount of hydroxyproline in the most soluble collagen fraction from the tenotomized, indomethacin treated tendons.

In another research by Carlstedt and (1987), the influence of indomethacin on biomechanical and biochemical properties of the plantaris longus tendon in the rabbit showed that there was a significant increase in tensile strength in the group treated with indomethacin. In one other study, Cottrell and O'Connor (2010), revealed that the use of NSAIDs as an analgesic is thought to negatively contribute to bone healing.

In another study, Thomas et al. (1991) revealed that indomethacin, and potentially other non-steroidal anti-inflammatory drugs, may be used in conjunction with a tendon injury without significantly affecting the early healing process. In the meantime, the use of NSAIDs in fracture patients should be cautious, keeping in mind the benefits of pain relief and inhibition of ectopic bone formation on one hand, and the risks of non-union and retarded union on the other hand (Vuolteenaho et al., 2008).

With comparison of different references, it was determined that indomethacin in low doses increases healing process and in high doses increases inflammatory phase of healing and in some cases yields gastric ulcers (Al-Bayaty et al., 2011; Nabavizadeh et al., 2011).

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