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Original article

Comparative effect of diclofenac sodium and dexamethasone on incisional wound healing in dogs

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ABSTRACT

The study was carried out to compare and evaluate the effect of diclofenac sodium, a non-steroidal anti-inflammatory drug (NSAID) and dexamethasone, a steroidal, anti-inflammatory drug on incisional wound healing in dogs. Fifteen (15) clinically healthy, mixed sex, intact Nigerian local dogs free of dermatological lesion with mean age of 14.97±3.7 months (Mean ± SD) and mean body weight of 10.73± 2.6kg (Mean ± SD) were used for the study. The dogs were randomly divided into three treatment groups: A, B and C comprising of five dogs per group. A caudal mid-ventral laparotomy skin incision was made to create surgical wounds. Immediately after anesthetic recovery, 7.5% diclofenac sodium injection intramuscularly at standard clinical therapeutic dose rate of 2.5mg kg^{-1} was administered to group A for three day, 2.5% dexamethasone injection intramuscularly at standard clinical therapeutic dose rate of 0.25mg kg^{-1} was administered to the group B for three days. Group C were not treated with any anti-inflammatory medication. Subjectively, diclofenac sodium group shows shorter healing interval compare to dexamethasone group ($P<0.05$). Objective histological evaluation at day 7 and 14 revealed low inflammatory density in group B compared to A and C, fibroblast, collagen fibers, and surface

keratinization was higher in group A at day 7 and 14 post surgery compared to B and C suggestive of faster healing in diclofenac sodium group compare to dexamethasone group. There was statistical significant different ($P<0.05$) among the groups. It is concluded that diclofenac sodium when used as an anti inflammatory agent post operative does not interfere with surgical wound healing.

1. Introduction

Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid hormones. It acts as an anti-inflammatory and immunosuppressant, its potency is about 20-30 times that of hydrocortisone and 4-5 times of prednisone. Dexamethasone can be used for a whole lot of purposes therapeutically, some of its therapeutic use include; Anti-inflammatory, oncologic uses, endocrine and obstetric purposes. It can also be used for diagnostic purposes (Cheville, 2006).

Dexamethasone is contraindicated in: existing gastrointestinal ulceration, cushing syndrome, severe forms of heart insufficiency, severe hypertension, uncontrolled diabetes mellitus, system tuberculosis, sever systemic viral, bacterial and fungal infections, it was hypothesized that it delayed wound healing by inhibiting mitotic cell division (Adams, 2001). Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) that works by reducing hormones that cause inflammation and pain in the body (Kumar *et al*., 2003). It is a phenyl-active derivative with different pharmacological features. It has anti-inflammatory, anti-pyretic and analgesic activities. Diclofenac sodium was developed specifically as an anti-inflammatory agent and has been used in the treatment of canine osteoarthritis (Aliu, 2007). It is well absorbed orally but undergoes subsequent first pass elimination, such that only 50% is available systematically. The drug is 99% protein bound, metabolized in the liver and excreted both in urine and bile. The plasma half-life is about 2hours. Diclofenac sodium has good tissue penetrability and accumulates in synovial fluid. The concentration in synovial fluid is maintained for 3 times longer period than in plasma, thus exerting extended therapeutic action in joints. The urine is the primary route of excretion for the drug and its metabolite (Aliu, 2007). Wound healing is one of the complex biological events after birth (Gillitzer *et al*., 2001). It is a complex process of the replacement of dead tissue by a vital tissue (Rubin *et al*., 1994). The response of the body to local injury begins very early in the process of inflammation and results in repair and regeneration. Regeneration is a replacement of injured tissue by parenchyma cells of the same type, sometimes leaving no residual trace of the previous injury (Kumar *et al*., 2003). Repair is a replacement by connective tissue, which in it permanent state constitutes a scar (Menetrey *et al*., 2000). The objective of the work was to compare and evaluate the effects of dexamethasone and diclofenac sodium on incisional wound healing using canine model.

2. Material and methods

Fifteen (15) clinically healthy intact male and female local Nigerian dogs were used for the study. The dogs' weights range from 7-14kg and ages between 7-12 months. They were kept in the small animal kennel of the Veterinary Teaching Hospital of the Usmanu Danfodiyo University. The animals were stabilized within two months, during which blood and fecal sample were collected for packed cells volume, complete blood count and determination of intestinal worn burden. They were treated with piperazine, multivitamin and iron dextran and fed with table remnants and water *ad libitum*. Surgical plane anesthesia was achieved with Xylazine 20 inj. (Xylazine HCl 20mg/ml) Kepro Holland at 0.5mgkg^{-1}; Atropine sulphate 0.6mg/ml (Laborate Pharmaceuticals India) at 0.05mgkg^{-1} intramuscularly as pre-anesthetic medications. 1% Propofol (Kepro Holland) at dose rate of 5mg kg^{-1} was used to induced and maintained anesthesia via catheterized cephalic vein after sedation. The animals were intubated with endotracheal and esophageal tubes for anaesthesia monitoring. The mid-ventral abdominal region was prepared for aseptic surgery according to standard procedure described by Charles *et al*., (1999). The dogs were placed on a dorsal recumbency; standard 10cm caudal mid-ventral laparotomy skin incision was made

through the linear alba. The laparotomy incision was routinely closed in two layer, the subcutaneous layer was closed with size 2/0 chromic catgut (Becton®) and the skin was closed with size 0 nylon (Agary®).

After anesthetic recovery, 0.2% Dexamethasone (Kepro Holland) at dose rate of 0.25mg kg^{-1} was administered intramuscularly to group B for three days. Group A were treated with diclofenac sodium (Laborate Pharmaceutical India) intramuscularly for three day at dose rate of 2.5mg kg^{-1}. Subjective evaluations of the healing interval and biopsy sampling were conducted at day 7 and 14 post surgery, biopsy samples was taken perpendicular to incision line and processed routinely for light microscopy (fixing, dehydrating, embedding, and cutting). Two sections were made from each sample and stained with hemotoxylin-eosin (H and E basic staining) and Masson's trichrome stain according to Gal *et al*., (2008). Sutures were removed at day 10 post surgery. The clinical appearance of the skin was scored twice: 18 to 24 hours and 10 to 14 days post surgery using the following criteria according to Sylvestra *et al*., (2002): swelling and erythema. Swelling and erythema distance were measured in millimeter away from the wound margin, in all cases measurement of 0, 0-2, 2-5 and >5(mm) were scored as 0, 1, 2 and 3 respectively.

Data obtained were tabulated; means and standard deviation were computed. One way analysis of variance (ANOVA) was used to compared statistical significant difference among the groups at 95% confidence interval using graphPad statistical soft ware package.

3. Results

At 18-24 hours post surgery, there was marked swelling and erythema in the control group; group B had the lowest swelling and erythema mean scores compared to group A indicating lower inflammatory response. There was statistical significant different of swelling (P<0.05) among the groups. However, there was no statistical difference of erythema even though the mean score varied among the groups (Table 1).

At 10-14 days post surgery, the swelling and erythema mean score were lower; group C had the highest swelling and erythema scores followed by A, group B had the lowest swelling and erythema scores (Table 1). There was no significant difference among the group (P>0.05).

Table 1
Mean wound score of different treatment groups at 18-24 hours and 10-14 days after surgery.

Outcomes	Total scores		
	Group A	Group B	Group C
18-24 hours			
Swelling	0.80 ± 0.25^{a}	0.50 ± 0.10^{b}	1.00 ± 0.20^{c}
Erythema	1.20 ± 0.50^{a}	0.70 ± 0.25^{a}	1.25 ± 0.40^{a}
Composite	2.00 ± 0.75^{a}	1.20 ± 0.35^{b}	2.25 ± 0.60^{c}
10-14 days			
Swelling	0.30 ± 0.10^{a}	0.10 ± 0.05^{a}	0.50 ± 0.10^{a}
Erythema	0.15 ± 0.05^{a}	0.10 ± 0.03^{a}	0.30 ± 0.10^{a}
Composite	0.45 ± 0.15^{a}	0.20 ± 0.08^{b}	0.80 ± 0.20^{c}

^{abc}Means on the same row with different superscript are significantly different (p<0.05).

The subjective healing interval showed that group A had lower mean healing interval in days with two days variation of healing interval compared to group B. The control group had the highest mean subjective healing interval of 18 days (Table 2). There was statistical significant difference among the group (P<0.05).

Table 2
Mean subjective healing interval of the group post surgery.

Groups	n	Mean healing interval
A	5	15.00 ± 1.50^{a}
B	5	17.00 ± 2.00^{b}
C	5	18.00 ± 1.50^{b}

^{ab}Means on the same Colum with different superscript are significantly different (P<0.05).

Histologic sectioning at 7 day post surgery revealed low inflammatory cells density in group B compared to A and C. The predominant cells were lymphocytes accounting for 90%, 75% and 80% of the inflammatory cells in group A, B and C respectively. Group A had higher granulation tissue, collagen fibers, fibroblast and epidermal keratinized cells at the wound surface compared to group B and C (Table 3; Plate I, II and III). At 14 day post surgery, there was no inflammatory cell observed in group B compared to group A and C with very mild inflammatory cells density. However, the density of granulation tissue, collagen fibers, fibroblast and epidermal keratinized cells were higher in group A compared to group B and C (Table 4; Plate IV, V and VI).

Table 3
Semi quantitative microscopic features of wounds in three groups at day 7

Parameters	Group A	Group B	Group C
Inflammatory cells density	++	+	+++
Inflammatory cells types	PMN (10%)	PMN (25%)	PMN (20%)
	Lymph (90%)	Lymph (75%)	Lymph (80%)
Granulation tissue formation	++++	+++	++
Collagen fiber density	+++	++	+
Fibroblast density	++++	++	++
Keratinization @ wound surface	+++	++	++

PMN= poly morpho nuclear; lymph= lymphocyte; + mild; ++ mild to moderate; +++ moderate; ++++ severe

Table 4
Semi quantitative microscopic features of wounds in three groups at day 14

Parameters	Group A	Group B	Group C
Inflammatory cells density	-/+	-	+
Inflammatory cells types	-	-	PMN (3%)
	-	Lymph (97%)	
Granulation tissue formation	-/+	+	++
Collagen fiber density	+++	++	+
Fibroblast density	+	++	+++
Keratinization @ wound surface	++	+	+

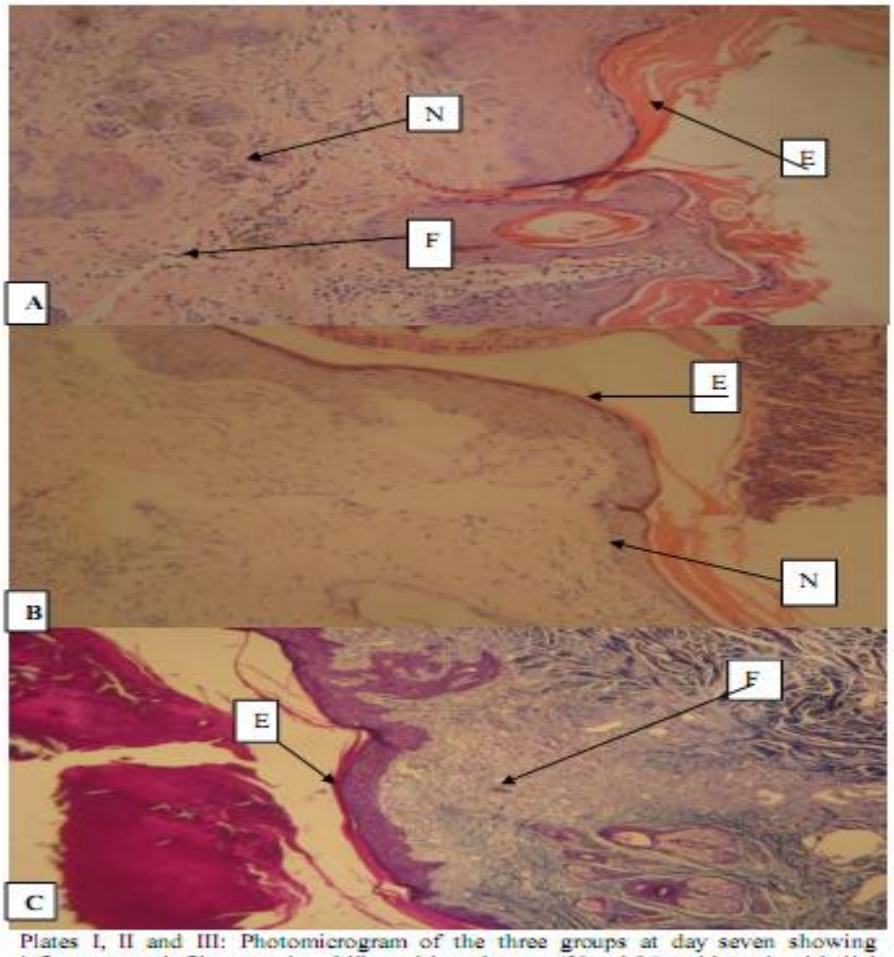
PMN= poly morpho nuclear; lymph= lymphocyte; -/+ very mild; + mild; ++ mild to moderate; +++ moderate; ++++ severe

4. Discussion

Dexamethasone has anti-inflammatory effects and has been used during operations for decreasing edema formation and swelling and for preventing ischemia-reperfusion injury (Henzi *et al*., 2000; Askar *et al*., 2002). Corticosteroids markedly affect most aspects of wound healing. When corticosteroids are administered early after injury, high corticosteroid levels delay the appearance of inflammatory cells and fibroblasts, the deposition of ground substance and collagen, regenerating capillaries, contraction, and epithelial migration (Erlich and Hunt, 1968; Wicke *et al*., 2000).

We found out that a triple dose of dexamethasone given to prevent postoperative inflammation has a deleterious effect on wound healing. The essential phase of wound healing is the inflammatory phase, characterized by increased vascular permeability, chemotaxis of the cells from circulation into the wound milieu, local release of cytokines and growth factors, and activation of migration cells (Witte and Barbul, 1997). In previous studies, corticosteroids reduced inflammation, which affects cell migration, proliferation, and angiogenesis (Leibovich and Ross, 1975). Corticosteroids inhibit the inflammatory phase, which causes delayed wound healing. Corticosteroids also inhibit collagen synthesis in wounded tissues and, therefore, have been used for treatment of corrosive esophageal burn to prevent stricture formation (Rappert *et al*., 1993). Corticosteroids also decrease collagen synthesis both in unwounded connective tissues and in fibroblast cell culture. The decrease of Type I collagen synthesis caused by steroids has been attributed to a decrease of the steady-state level of total cellular Type I procollagen messenger RNAs. Glucocorticoids regulate α^2-Type I procollagen promoter activity (Perez *et al*., 1992). The wound-healing process has been conveniently divided into three phases; inflammatory, proliferative, and remodeling. However, the process is continuous, and phases overlap. Therefore, the conceptual

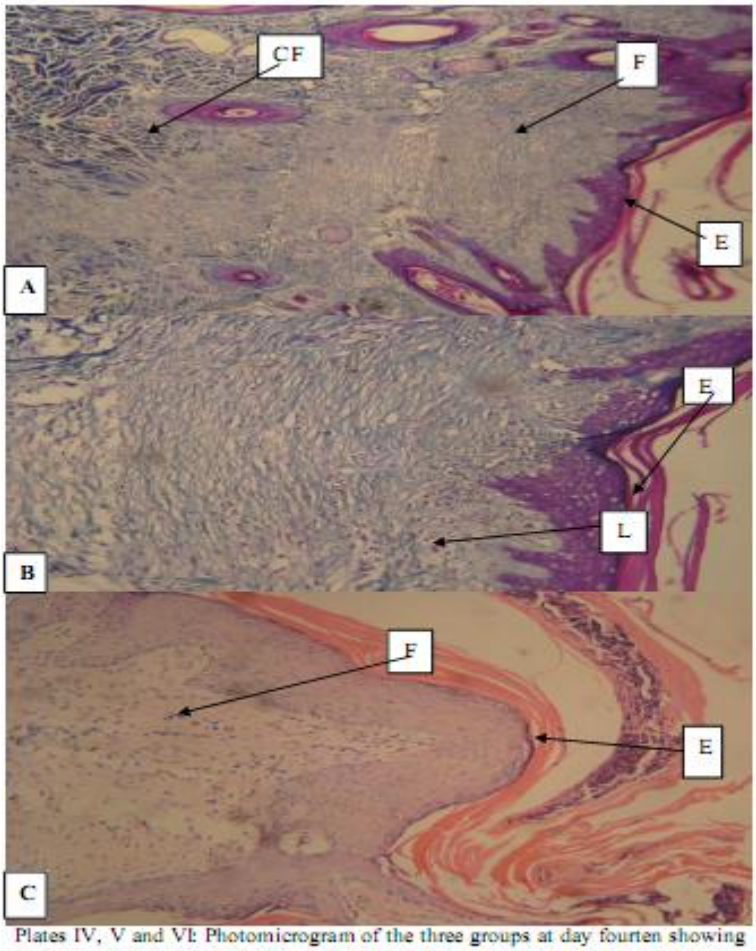
distinction between phases serves only as an outline to discuss events that occur during wound repair. The presence of more mature capillary vessels in the vicinity of a wound allows for better nutrition, and this phenomenon, combined with a large amount of collagen fiber, is directly related to a more adequate wound healing process (Drucker *et al*., 1998). Angiogenesis is a dynamic process during wound healing, as the fibrin clot is replaced by blood vessel-rich granulation tissue and is subsequently replaced by a collagenous scar with much less mature vessels (Clark *et al*., 1982).



Plates I, II and III: Photomicrogram of the three groups at day seven showing inflammatory infiltrates; nitrophills and lymphocyte (N and L), epidermal epithelial keratinization at the wound surface (E) and fibrolast(F). A and B stained with H and E and C with Masson,s tricrome, X400 objective magnification

In our study, there were significantly less inflammatory cells and vascularity in the dexamethasone group. The presence of significant less inflammatory cells and vascularity in the dexamethasone group compared with the diclofenac sodium and control group might be related to delayed inflammatory and proliferation phases. Increased collagenization and epithelization in diclofenac sodium group with high inflammatory cells and high vascularity provided evidence of repletion of granulation tissue to collagenous scar in the control group (Tekin *et al*., 2000).

We performed a comprehensive histological analysis of wounds of dexamethasone and diclofenac sodium treated animals using the semi quantitative assessment strategy described here. As summarized in the histological example presented in table III and IV and plates I-VI at 7 and 14 days post wounding, diclofenac sodium treated wounds exhibit an overabundance of proliferating epidermis at the wound edges; migration of subsequent re-epithelialization, higher fibroblast and collagen density; suggestive of faster healing compared to dexaethasone treated wound. On the other hand the dexamethasone treated wounds were characterized with lower inflammatory infiltrates at both day 7 and 14 post wounding but failed to show adequate fibroblast, collagen fiber density and epidermal keratinization; suggestive of slower healing progress compared to diclofenac treated animals.



Plates IV, V and VI: Photomicrogram of the three groups at day fourten showing varying degree of inflammatory infiltrates (**N** and **L**), collagen fiber (**CF**), fibroblast (**F**) and epidermal epitalization (**E**). C stained with H and E and A and B with Masson,s tricrome, X400 objective magnification

5. Conclusion

In conclusion, this study has shown that dexamethasone at 0.25 $mgkg^{-1}$ doses may have negative effects on wound healing. To substantiate the dose-related effects, further investigations with dexamethasone at different doses will be required. It was also concluded that diclofenac sodium at 2.5 $mgkg^{-1}$ dose does not have negative effects on wound healing.

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