EFFECTS OF THE DICLOFENAC SODIUM (NSAID) ON COLLAGEN CONNECTIVE TISSUE FIBRES IN CAECAL MUCOSA OF ALBINO RATS

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ABSTRACT
To evaluate the effects of diclofenac sodium (NSAID) on Collagen connective tissue fibers of albino rats. An animal study carried out in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi. Diclofenac sodium was administered to male albino rats at a dose of 2 mg/kg body weight orally once daily for two weeks. These animals were sacrificed, Caeca were identified and removed, opened along mesenteric border, fixed in alcoholic formalin, Embedded in paraplast, 4 um thick sections were cut on rotary microtome, stained with Mallory’s trichrome Connective tissue stain. The histomorphological features of caecal mucosa were compared with those in the control animals. The study revealed that diclofenac sodium administration produced and increased amount of Collagen connective tissue fibers forming a sub-epithelial collagen band in caecal mucosa of albino Rats. The results suggest that diclofenac sodium causes. Severe caecal mucosal damage in albino rats.

Keywords: Diclofenac sodium, Caecum, Collagen Fibers, albino rat.

INTRODUCTION

Diclofenac is an anti-inflammatory agent approved for several uses in the United States as a sodium or potassium salt. It is a benzene acetic acid derivative, designated chemically as 2-[2,6—dichlorophenyl amino benzene acetic acid] mono-sodium or mono-potassium salts. It is a faintly yellowish white to light beige virtually odorless, Slightly hygroscopic crystalline powder. It is recommended for the long term treatment in Rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and for short term treatment in renal colic, acute gout, acute musculoskeletal injury, acute painful shoulder, Postoperative pain, migraine, and dysmenorrhoea. In addition, an Ophthalmic solution after cataract extraction (Gillman et al., 2001).

Although the drug diclofenac sodium is contraindicated in patients who have experienced asthma, urticaria or other allergic type reactions, G.I.T disturbances, hepatic Insufficiency, renal impairment, and pregnancy (Arky, 1997). But quacks are liberally using the drug unchecked in general clinical practice in our population, which may be prohibited by the Government under rules.

This study was done to evaluate the effects of diclofenac sodium (NSAID) on Caecal collagen connective tissue fibers of albino rats.

MATERIALS AND METHODS

Eighty albino rats were used in this study, which obtained from Animal House of Basic
Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. All were male, 20 weeks of age, weighing 180-200 grams, looking active and healthy. These Animals were housed in the experimental room of Animal House maintained on balanced Laboratory diet and water ad libitum with 12 hours light and dark cycle.

Eighty animals were divided into two equal groups; A and B, Each comprising of 40 animals.

- Group -A animals were given diclofenac sodium (developed in Novartis Pharma Pakistan Ltd) at a therapeutic dose of 2 mg/kg body weight orally once daily for 2 weeks (Manocha and Venkataraman 2000).
- Group- B animals used as control and were given normal saline (equal volume of dose given to group- A) orally once daily for 2 weeks.

All the rats were sacrificed on day-15 of the experiment by giving Deep Ether anesthesia and were operated to obtain their caeca, which were fixed in Alcoholic formalin, embedded in paraplast and 4 um thick Sections were cut on Rotary microtome. These sections were stained with Mallory’s trichrome Connective tissue stain. The histomorphological features of caeca in both Groups were observed with respect to collagen Connective tissue fibers.

The status of collagen connective tissue fibers was observed and Recorded as +(slight), ++ (moderate), and +++ (marked).

RESULTS

General Observations
The animals in group- A looked slow and weak during last 2-3 days of Experimental period. They appeared lethargic, their response to stimuli was Sluggish and food intake was decreased as compared to animals of Group-B.

Microscopic Observations
Under laboratory microscope the animals of Group-A showed inflammatory Exudates including numerous lymphocytes, plasma cells and neutrophils were scanty to negligible observed in almost all of the animals and the collagen connective tissue fibers Showed marked quantity with thickened sub epithelial band of collagen, which were irregularly arranged, as shown in Fig. 1.

![Fig. 1. Photomicrograph of 4 um thick paraplast section of caecum stained with Mallory's connective tissue stain in diclofenac sodium treated (group-A) albino rat, showing increased collagen fibers, forming a sub epithelial collagen band, marked against arrows, under high power objective. x416.](image-url)
DISCUSSION

The present study was designed to observe the morphological effects of Diclofenac sodium (NSAID) on collagen connective tissue fibers present in caecal Mucosa of albino rats.

The diclofenac sodium administered in a normal therapeutic dose of 2 mg/kg body weight, once daily orally for 2 weeks (Manocha and Venkataraman 2000) produced exfoliation and depletion of epithelial cells of caecal mucosa and increased amount of collagen connective tissue fibers running irregularly and forming sub epithelial thick band.

After treatment with diclofenac sodium (NSAID) in animals of group-A, general Behavior changed to ill, sluggish and decreased food intake which may be attributed to Unwanted effects of diclofenac sodium toxicity. In this context our results are in Agreement with Gabriel et al (1991), Bjarnason et al (1993), and Graham et al (1993) who stated that Administration of diclofenac sodium was associated with increased gastrointestinal Toxicity include mild dyspepsia or cachexia as well as more serious gastrointestinal Reactions such as ulceration, bleeding, perforation and other events leading to Hospitalization or death.

On microscopic examination of caecum revealed decreased mucosal thickness and increased sub epithelial collagen fibres. These changes are in conformity with the Studies by Van-kolfshoten et al. (1983), Kaufman and Taubin (1987), Graham et al (1993), and Manocha and Venkataraman (2000). In those studies.

The Investigators found common mucosal lesions, i.e. erosions and ulcers found in Stomach, Small and large intestines except caecum. A highly significant decrease in mucosal thickness was observed which may be attributed to the injurious effect caused by diclofenac sodium (NSAID) which might have resulted into onset of the demolition with extensive exfoliation of surface epithelial cells and ulceration, mucosal lining of caecum showed necrosis which according to Kumar et al (1989) resulted most commonly from sudden severe Ischemia due to irreversible injury to cells. Inflammatory necrosis.

Associated with intake of NSAID along with marked Infiltration of lymphocytes, Plasma cells, apoptotic, pyknotic, flattened cells, and neutrophils were noticed in lamina propria as well as within epithelium. Our findings are in agreement with Kumar et al (1989) and Lee (1993) who found that the presence of apoptotic Bodies especially in the colonic crypts might indicative to exposure in particular to NSAID therapy.

Apoptosis was found to be a conspicuous feature in cases of colitis related beyond reasonable doubt to the administration of

Fig. 2. Photomicrograph of 4 um thick paraplast section of caecum stained with Mallory's connective tissue stain in (group-B) albino rat, showing normal amount of collagen fibers in sub epithelium under high power objective. x416.
diclofenac sodium. Apoptotic Bodies were present in substantial number in colitis associated with diclofenac sodium.

The mechanisms by which drugs bring about apoptotic changes are far from clear. In NSAID associated colitis, cryptal apoptosis have been frequently accompanied by other histological abnormalities, more notably an increase in lymphocytes in lamina propria as well as increase in intraepithelial lymphocytes mainly in crypts themselves. In some instances there has been evidence of more acute Crypt damage with incipient or even frank crypt abscess formation. In the case of NSAID associated lesions, however, apoptosis have been accompanied by inflammatory changes and in particular by a focal increase in intraepithelial lymphocytes in the crypts and may well be immunologically mediated (Lee, 1993). The collagen fibers showed a highly significant increase forming a sub-epithelial collagen band in lamina propria; this may be attributed to inflammatory allergic process due to injurious effect by diclofenac sodium. Our results are in agreement with Kumar et al (1989), Giardello (1990), and Riddell et al (1992) who found that immediate sub epithelial zone showed the deposition of hypo cellular collagenous band in lamina propria and in histologic.

Hallmark in collagenous colitis following use of NSAID. The mechanism by which NSAID causes colitis and sub epithelial collagen band thickening remains unclear, However, it may be suggested that in the large bowel as well as the small, NSAID opens the Para cellular pathway at least sufficiently for the luminal contents to enter the lamina propria, thereby inducing mucosal inflammation. Although this is also the site of collagen band thickening, local abnormalities of collagen synthesis related to the peri-kryptal fibroblastic sheath and decreased cell turnover time have been considered as another Possible mechanism of collagen band thickening.

CONCLUSION

These results suggest that diclofenac sodium causes severe caecal mucosal damage in albino rats.

REFERENCES


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