COMPARATIVE ADVERSE EFFECTS OF COX-1 AND COX-2 INHIBITORS IN RAT LIVER : AN EXPERIMENTAL STUDY."

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ABSTRACT

The non-steroidal anti-inflammatory drugs (NSAIDs) do not reverse the disease process, but they provide much needed relief from pain and inflammation by inhibiting cyclooxygenase (COX) enzyme mediating the inflammatory pathway. NSAIDs cause number of death as a result of upper gastrointestinal damage. These agents also have unwanted effects on lower bowel, lungs, kidney and cardiovascular symptom. Coxibs are selective inhibitor of cyclooxygenase (COX)-2 and spares the COX-1 induced side effects i.e. gastric ulceration. Therefore in present study we compared the adverse effects of diclofenac sodium (diclo) a non-selective NSAID and valdecoxib (valde), a selective NSAID at therapeutic and subtherapeutic doses. Histological and biochemical changes of liver were observed. Beside that gross examination of the stomach mucosa for ulceration along lesser curvature too observed. For liver functions we estimated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP). Experiment was carried out by administration of drugs for period of 30 days. Liver sections of diclo & valde groups showed histological changes, which were more prominent with therapeutic dose of valde. The biochemical changes, subtherapeutic dose of diclo showed increase in ALP only. On the other hand subtherapeutic dose of valde showed significant changes in AST, high significant changes in LDH & ALP. Whereas therapeutic doses of diclo and valde showed highly significant increase in hepatic biochemical parameters i.e. AST, ALT, ALP, LDH.

Thus it may conclude that higher doses of COX-1&2 inhibitors can lead to acute hepatitis and other hepatic complications.

Keywords: NSAIDs, coxibs, liver, stomach

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) reduce pain and improve function in people with mechanical and inflammatory arthropathy but these benefits come at a price. Every year NSAIDs cause number of deaths as a result of upper gastro intestinal damage. These agents can also have unwanted effects on the lower bowel, lungs, kidney and cardiovascular system. Recent trend is for selective NSAIDs, which were launched, in early 1999, as an alternative to traditional non-steroidal anti-inflammatory drugs (NSAIDs). The cyclooxygenase (COX) enzyme exists in two isoforms (Needleman & Isakson 1997)[1]; a constitutive isoform is COX-1 mainly associated with housekeeping function and its antagonism, resulted into gastric ulceration. Whereas inducible isoform is COX-2 and its antagonism thus provides relieve from pain and inflammation (Masferrer et al.1994[2], Seibert et al. 1994[3] and Warner et al. 1999)[4].

COX-2 selective agents have been increased by the addition of valdecoxib, etoricoxib and lumiracoxib. These COX-2-selective drugs together with the NSAIDs cover a wide range of selectivity toward COX-1 and COX-2 (Van Ryn et al. 2000)[5]. COX-2 selective inhibitors appear to be associated with less gastrointestinal damage than conventional NSAIDs (Simon LS 1997)[6]. Further, valdecoxib has been shown to cause substantially insignificant gastroduodenal ulcers than NSAIDs while the efficacy of the two groups is equal (Sikes et al. 2002)[7].

The hepatic injury associated with NSAIDs observed in clinical trials was found to be quite variable ranging from mild cholestasis to severe hepatocellular injury along with biochemical changes (Lewis et al. 1996[8] & Zimmerman HJ 1996)[9] that is often not clinically relevant and returns normal upon cessation of treatment. Selective COX-2 inhibitors were also associated with hepatotoxicity (Merlani et al. 2001[10] and Algeria et al. 2002)[11].

The present study was conducted

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to observe the adverse outcome of diclo and valde in stomach and liver of albino rats. The effects of diclo (non-selective NSAID) and valde (selective NSAID) were compared and assessed for gross outcome in stomach and histological along with biochemical changes in liver at therapeutic and sub-therapeutic doses in rats.

MATERIAL AND METHODS

Animals

50 Adult, 25 male & 25 female Dukrey rats (180-200gm) were obtained from National Animals Laboratory Centre, Central Drug and Research Institute (CDRI), Lucknow. Experimental protocols were approved by Institutional Ethical Committee following the guideline of CPSCEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Drugs

Diclofenac sodium and Valdecoxib were obtained from M/s Karnataka Antibiotic Pharmaceutical Ltd. and Glenmark Pharmaceuticals Ltd. respectively.

Enzyme Estimation

E-merk's diagnostic kits were used for the estimation of enzyme - AST, ALT, ALP and LDH related to liver function.

Dosage and administration

The doses administered in this study, were calculated according to the therapeutic dose as recommended for the chronic treatment of arthritis. We gave diclo at the doses of 5 and 10 mg/kg/day whereas valde at the doses of 1 and 2 mg/kg/day per orally. The subtherapeutic dose, half of the therapeutic dose was selected to see the level at which changes begins.

Methods

(a) Rats of either sex were randomly divided into 5 groups of

10 (5 male and 5 female) each and drugs were administered per orally for 30 days as follows:
Group A - served as control was administered with normal saline only
Group B1 - Diclofenac sod. 5 mg / kg / day p.o.
Group B2 - Diclofenac sod. 10 mg / kg / day p.o.
Group C1 - Valecoxib 1 mg / kg /day p.o.
Group C2 - Valdecoxib 2 mg / kg/ day p.o.

Drugs were freshly prepared daily by dissolving in normal saline before oral administration.

(b) Blood sample - was collected after the end of treatment i.e. on day 30, from left ventricle of rats.

(c) Specimen collection - the control and treated group of animals were sacrificed 30 th day post treatment. Animals were perfused with 10% formalin and 0.9% normal saline. The sections of the organs were stained using H/E (hematoxylin and eosin) and viewed under 40X magnification.

(d) Stomach mucosal lesion of rats were seen only in unperfused rats. Stomach was opened along lesser curvature. The inner surface was then everted for full internal examination.

(e) Statistical analysis data of biochemical parameters were presented as mean + SEM (Standard Error of Mean) for control and experimental animals. Statistical significance was set at $p<0.05$.

OBSERVATION

STOMACH MUCOSA:

No epithelial shedding and hemorrhagic lesions were seen in any of the treated group as compared to control group (Fig: 1).

LIVER:

Histological changes

The histopathological sections of liver of group B1, treated with subtherapeutic dose diclo, showed enlarged hepatocytes with some vacuolations in the cytoplasm. Sinusoidal spaces were reduced along with congested vessels in the interstitium (fig:2b) as compared to control group A (fig:2a). Group C1, treated with subtherapeutic dose valde, showed enlarged hepatocytes with cytoplasmic blebbing. Few binucleated cells were also seen. Central vein showed dilatation (fig: 2c). Periportal lymphocytic infiltration also seen with other feature (fig: 2d). Group B2, treated with therapeutic dose diclo was associated with more prominent changes as compared to group B1. Enlarged hepatocytes with abundant vacuolation were observed (fig: 2e). Group C2, treated with therapeutic dose valde showed more prominent changes as seen with group C1 (fig: 2f) in comparison to control groupA Biochemical changes

AST showed significant increase in groups C2 ($p<0.001$), B2 and C1 ($p<0.05$) on 30th day when compared to control group. ALT showed significantly increased level in groups B2 ($p<0.001$) and C2 ($p<0.01$). ALP and LDH showed highly significant increase in group B2, C1 and C2 ($p<0.001$) (Table-I). There was no significant difference in the biochemical parameters of male and female rats of

Group (Drug/Dose mg/kg p.o.)	S.ALT (U/L)	S.AST (U/L)	S.LDH (U/L)	S.ALP (U/L)
A (Control)	35.93± 6.45	126.86± 9.38	614.34± 317.16	260.55± 73.17
B_1 (Diclofenac, 5)	31.65+ 4.74	116.17+ 20.70	624.23+ 307.12	510.11+ 138.99***
B_2 (Diclofenac, 10)	52.01± 10.032***	150.77± 17.06**	1206.10± 92.63***	759.61± 93.08***
C_1 (Valdecoxib, 1)	32.70+ 7.72	146.24+ 22.41*	900.08+ 343.72***	711.70+ 91.28***
C_2 (Valdecoxib, 2)	47.26± 9.087**	203.90± 39.49***	1258.20± 111.68***	711.75± 91.20***

Table I: Results of hepatic biochemical parameters

#The values are expressed as mean standard error. *p<0.05; **p<0.01; ***p<0.001 as compared to control. (U/L= unit per liter)

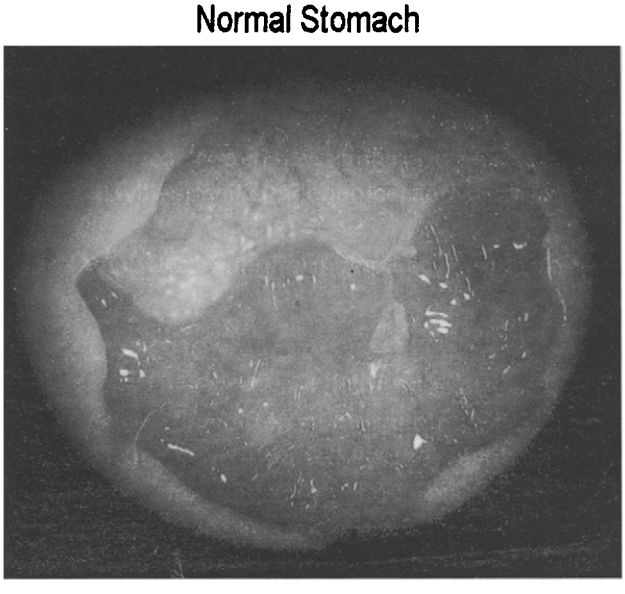

Fig:1a Showing internal surface of stomach with no epithelial shedding and hemorrhagic lesion

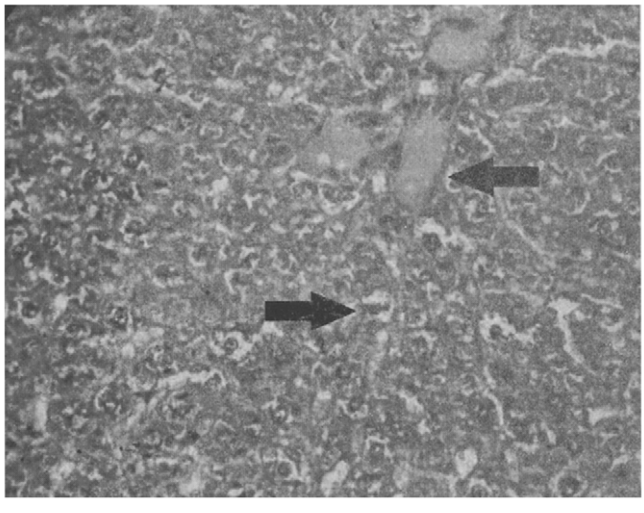

Fig2b: Liver of group B1 (Diclo 5mg/kg), small arrow showing enlarged hepatocyte and large arrow shows congested vessels (40X)

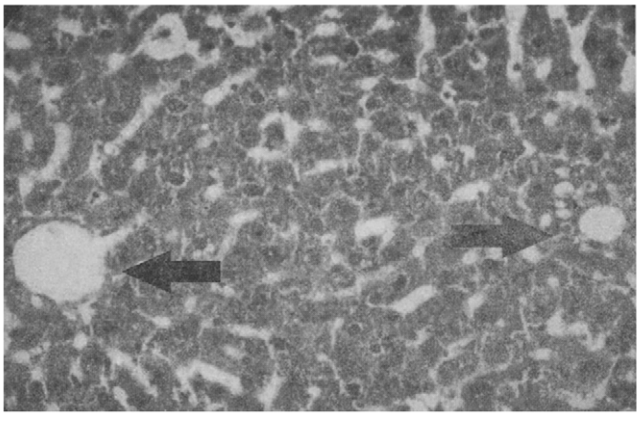

Fig 2a: Liver of control group A, large arrow showing central vein and small arrow showing portal triad (40X)

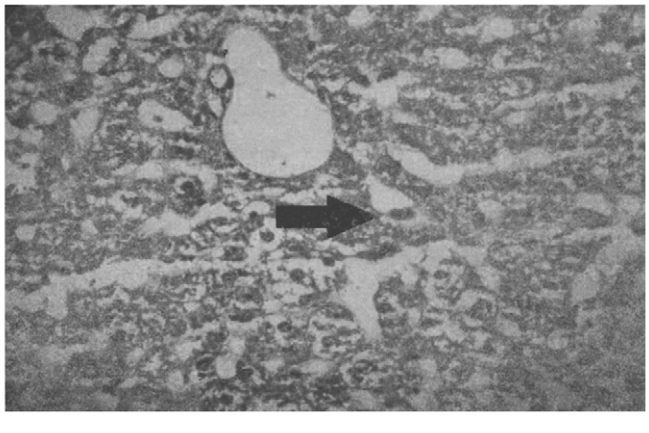

Fig2c: Group C1 (valde 1mg/kg), arrow showing enlarged hepatocyte with blebbing (40X)

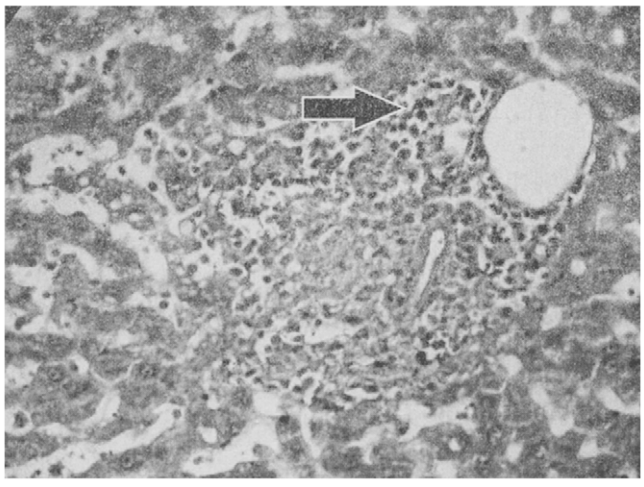

Fig 2d: Group C1 (valde 1mg/kg), arrow showing periportal lymphocytic infiltration. (40X)

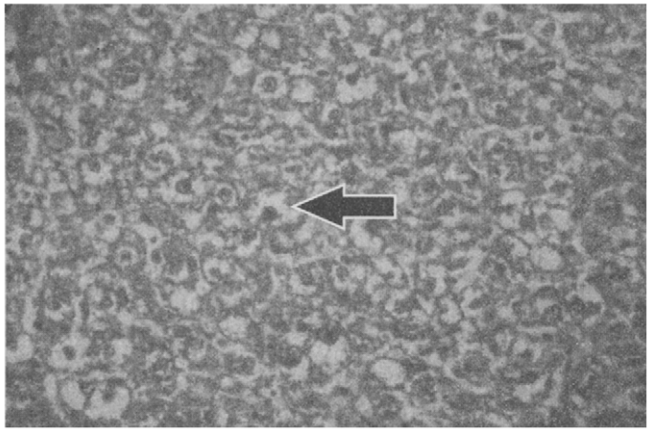

Fig 2e: Group B2 (Diclo 5mg/kg), arrow showing vacuolated hepatocyte(40X)

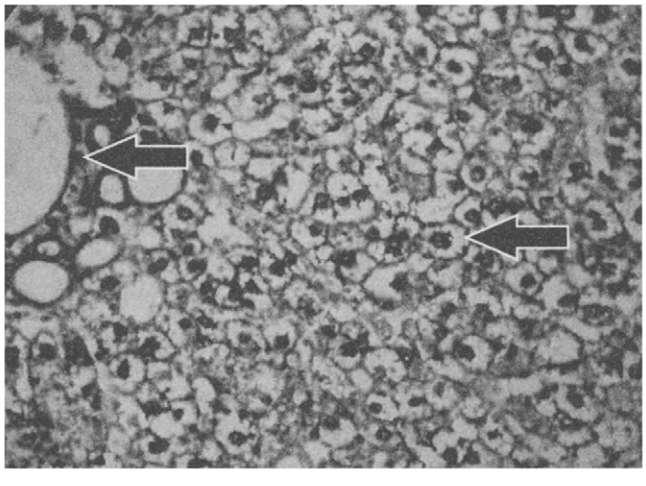

Fig2f: Group C2 (valde 2mg/kg), large arrow showing dilated central vein and small arrow enlarged hepatocyte (40X)

each group.

DISCUSSION

Previous data states that selective COX-2 inhibitors induces significantly less gastric damage than traditional NSAIDs; both in animals and humans (Penning et al. 1997[12], Chan et al 1999[13], Hawkey CJ 1999[14], Masferrer et al 1994[2], Laine L 2002)[15]. In the present study, it was found that stomach mucosa of the groups receiving diclofenac were absolutely free from any epithelial damage or hemorrhagic ulceration in both sexes. It was found contrary to earlier studies probably due to the acute exposure (1 month) in healthy gastric mucosa might be resistant to the damage. As expected, both groups of valdecoxib supported the earlier studies and did not show any damage in gastric mucosa.

Hepatic dysfunction has been associated with the clinical use of NSAIDs including diclofenac. This class of drugs causes borderline increase in the levels of liver enzymes in patients taking drugs regularly (Maddrey et al 2000)[16]. Present study with high dose of diclofenac repeated the result of previous studies in histological and biochemical parameters observed by Manocha & Venkatraman (2000) with diclofenac (2mg/kg) and nimesulide (4mg/kg) for 14 days in wistar rat. Burden et al (2004)[17] in rats with ibuprofen (8.5mg/kg) selective NSAIDs DFU (0.2mg/kg) and Prakashreddy et al. (2006)[18] with nimesulide (2 & 5 mg/kg) and diclofenac (5 mg/kg) for 28 days in birds. Since subtherapeutic dose of diclofenac showed histological changes with normal ALT, AST and LDH levels. Therefore at subtherapeutic dose only histological changes were observed.

Present study Valdecoxib at subtherapeutic as well as therapeutic dose showed necrosis of hepatocytes and inflammatory infiltrate along with raised liver enzymes. Nachimuthu et al. (2001)[19] and O'Beirne and Cairns SR (2001)[20] had similar observation with celecoxib in clinical trials. Burden et al (2004)[17] in pregnant & non pregnant rats on other hand contradictory to present study none of the changes, histological or biochemical parameter of liver with nimesulide (selective NSAIDs) reported by Barkoskava et al. (2004)[21] in clinical trials and Prakash reddy et al (2006)[18] with doses 2 & 5 mg/kg in birds.

The results of the present study add to distinct heterogeneity within widely prescribed class of drugs. Thus, sub-therapeutic and therapeutic doses of valdecoxib, on long-term exposure can lead to hepatic dysfunction or acute hepatitis. Whereas with

diclofenac, it therapeutic dose can lead to pathological changes while its sub therapeutic dose seems to be safer than valdecoxib in hepatic outcome. Thus, it can be inferred that changes begin at subtherapeutic level. Therefore these drugs need to be taken with precaution in patient of hepatic compromised conditions.

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