Original Article

Comparative Study of Gastric Tolerability and Anti-Inflammatory Activity of Various Nsaids in Rats

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ABSTRACT

Ulcers can also be caused and worsened by drugs such as aspirin, ibuprofen and other NSAIDs. Irrational usage of NSAIDs leads to the ulceration of the stomach and sometimes found to cause perforations due to the lack of certain prostaglandin synthesis which are essential for the production of gastric mucosa. Purpose of present study was to evaluate the acute damage caused by different dosages of three NSAIDs; diclofenac sodium, mefanamic acid and piroxicam in rats to provide information for understanding the mechanism underlying acute NSAIDs- induced gastric damage. Healthy wister rats weighing 200- 250 grams were used for gastric tolerability test. Gastric tolerability of NSAIDs was determined by the pylorus ligation model and their anti-inflammatory activity was determined by carragennan induced acute paw oedema model. All of the NSAID groups showed significantly higher gross ulcer index values than the control group. The gross ulcer index increased with alone and combination of the NSAIDs in rats. Mefanamic acid treatment group and its diclofenac sodium combination showed less ulcer index when compared to other treatments. That is, the gross ulcer index in combination of Diclofenac sodium and Piroxicam was significantly higher than that of control and individual treatments. All the groups of NSAIDs showed good anti-inflammatory action. Combination of piroxicam and diclofenac sodium showed high anti-inflammatory activity compared to individual treatments. From the above results we can conclude that the combination of diclofenac sodium and piroxicam showed additive anti-inflammatory activity. Combination of diclofenac sodium and mefanamic acid was found to be well tolerated with gastric mucosa.

Keywords: NSAIDs, Pylorus ligation, Carragennan, Prostaglandins.

INTRODUCTION

A history of heartburn, gastro esophageal reflux disease and use of certain forms of medication can raise the suspicion for peptic ulcer. A peptic ulcer, also known as peptic ulcer disease[1] (PUD) is the most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. Ulcers can also be caused and worsened by drugs such as aspirin, ibuprofen and other NSAIDs.

Helicobacter pylori may cause ulcers. In the Treatment of H-pylori infection usually leads to clearing of infection, relief of symptoms and eventual healing of ulcers. Recurrence of infection can occur and retreatment may be required, if necessary with other antibiotics. Omeprazole, rabeprazole are the drug of choice in the treatment of ulcers. Perforated peptic ulcer is a surgical emergency and requires surgical repair of the perforation. Most bleeding ulcers require endoscopy urgently to stop bleeding with cautery, injection, or clipping[2].

Ranitidine and Famotidine, which are both H2 antagonists, provide relief of peptic ulcers, heartburn, indigestion and excess stomach acid and prevention of these symptoms associated with excessive consumption of food and drink. Ranitidine and famotidine are available over the counter at pharmacies, both as brand-name drugs and as generics, and work by decreasing the amount of acid the stomach produces allowing healing of ulcers. Sucralfate (Carafate) has also been a successful treatment of peptic ulcers.

The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs found to block the function of cyclooxygenase-1 (COX-1), which is essential for the production of these prostaglandins. COX-2 selective anti-inflammatory drugs (such as celecoxib or since withdrawn rofecoxib) preferentially inhibit COX-2, which is less essential in the gastric mucosa, and roughly have the risk of NSAID-related gastric ulceration[3]. Irrational usage of NSAIDs leads to the ulceration of the stomach and sometimes found to cause perforations.

It has been reported that gastric toxicity is strongly influenced by the amount of drug dissolved under the pH conditions rather than the potency of the drug as an inhibitor of prostaglandin synthesis[4]. For example, mefanamic acid exerts its gastrotoxic effects predominantly by localized action during the gastric absorption of the drug; In contrast, piroxicam is associated with greater intestinal toxicity compared to other NSAIDs, suggesting that it could be related to enterohepatic circulation and the continuous inhibition of prostaglandins[5]. These results suggest that the extent of ulcer damage and the related mechanisms might be different depending on the type or dose of NSAID.

Based on these previous findings, we investigated the acute damage caused by different dosages of three NSAIDs; diclofenac sodium, mefanamic acid and piroxicam[6] in rats to provide information for understanding the mechanism underlying acute NSAIDs- induced gastric damage.

MATERIALS AND METHODS

Animal

Healthy wister rats weighing 200- 250 grams were used for gastric tolerability test. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (liptonindia laboratories, Bangalore) and water *ad libitum* and were fasted overnight before the day of experiment. Animals were housed within the departmental animal house, and the room temperature was

maintained at 27 degrees Celsius[7]. The study was approved by the institutional animal ethical committee (EC/2013/14).

Investigational Drugs and Dosage preparation

Tablet Diclofenac sodium (Korten Pharmaceuticals Pvt.Ltd, Thane) was purchased from the hospital pharmacy counter. Tablet Piroxicam(Cipla Ltd,E-65/66 Midc, Solapur) and Mefanamic acid (Blue Cross Laboratories Ltd, L-17, Goa) was also procured from the same hospital outlet. The appropriate body weight adjusted doses of test drugs as extrapolated from doses used in similar studies were used.

Formulations were made as suspension prepared in gum acacia 2% w/v uniformly mixed. The formulations were fed to animals through gastric tube (9 mm) for rats and 2 – 3 cms polythene tubing sleeved on an 18-20 gauge blunted hypodermic needle for rats. The vehicle gum acacia (2% suspension) alone was used as a control in all the groups[8].

Experimental design

The Animals (n=30) were allocated to 6 groups (GC, GD, GP, GM, G DP, G DM) of 5 animals each. Groups of five animals each receiving gum acacia as the control (GC), diclofenac sodium 10mg/kg,i.p (GD), mefanamic acid 30mg/kg,i.p (GM), piroxicam 10mg/kg,i.p (GP), combination of diclofenac sodium and mefanamic acid (G DM), and combination of diclofenac sodium and piroxicam (G DP) respectively 30 min prior to pyloric ligation.

Pylorus- ligation induced gastric ulcer

Animals were anesthetized with ether and stomach exposed with small incision. Thread passed around the pyloric sphincter and applied a tight knot[9]. After 4 hr of pyloric ligation, animals were sacrificed by decapitation method. Stomach was removed to collect the gastric contents. The mucosal surface was macroscopically observed and ulcer scores were determined.

Score the ulcers as below:

0= normal colored stomach
0.5=red coloration
1= spot ulcers
1.5= heamatologic streaks
2= ulcers≥3 but≤5
3=ulcers ≥5

The total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatent liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour[10&11]. The volume of NaOH required was noted and was taken as corresponding to the total acidity.

Acidity was expressed as: Acidity = Volume of NaOH x Normality x 100 / 0.1

Carragennan induced paw oedema model in

Animals were numberd and marked on both the hind paws (right and left) just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume[12]. The initial paw volume (both right and left) of each rat by mercury displacement method was noted for all groups. After 30min injected 0.1ml of 1% (w/v) carrageenan in the plantar region of the left paw of control as well as treated group. The right paw will serve as reference non-inflammaed paw for comparison. The paw volume of both legs of control and treated noted at 1hr, 3 hr and 6hr after carrageenan challenge[13&14]. Calculated the percent difference in the right and left paw volumes of each animal of control and treated groups. Compared the mean per cent change in paw volume in control and treated animals

and expressed as percent oedema inhibition by the drug.

Statistical analysis

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analyzed separately and one-way analysis of variance was carried out[15].

RESULTS & DISCUSSION

The gross appearance of gastric damage patterns was different depending on the type of NSAIDs, and became more apparent when the damage was severe. Diclofenac sodium caused a punctuate type clean eruptions. The gastric damage induced by Piroxicam was similar to that caused by Dicolfenac and Mefanamic acid[16], but the depth of ulcers was shallow and the damage extent was less severe than that caused by combination of Diclofenac sodium and Piroxicam. In contrast, the ulcers caused by the combination groups were severe when compared with that of treatment alone, especially combination of Diclofenac sodium and Piroxicam.

All of the NSAID groups showed significantly higher gross ulcer index values than the control group. The gross ulcer index increased with alone and combination of the NSAIDs in rats. Mefanamic acid treatment group and its diclofenac sodium combination showed less ulcer index when compared to other treatments[17&18]. That is, the gross ulcer index in combination of Diclofenac sodium and Piroxicam was significantly higher than that of control and individual treatments (figure 1).

All the groups of NSAIDs showed good anti-inflammatory action[19]. Combination of piroxicam and diclofenac sodium showed high anti-inflammatory activity compared to individual treatments (table 1).

CONCLUSION

From the above results we can conclude that the combination of diclofenac sodium and piroxicam showed additive anti-inflammatory activity. Combination of diclofenac sodium and mefanamic acid was found to be well tolerated with gastric mucosa when compared to that of control group[20]. Biochemical and acute toxicity studies to be carried out to determine the efficacy and safety of the usage of these drugs in combination.

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Table 1. Anti-inflammatory activity of NSAIDs

GROUPS	Change in paw volume (ml) mean±SEM& % inhibition		
	1 hr	3 hr	6 hr
Control	0.52±0.02	0.61±0.02	0.65±0.03
Diclofenac sodium	0.24±0.02 (43.08)	0.36±0.02 (40.98)	0.24±0.02 (63.08)
Mefanamic acid	0.27±0.02 (48.08)	0.41±0.01 (32.79)	0.22±0.01 (66.15)
Piroxicam	0.21±0.03 (59.62)	0.42±0.01 (31.15)	0.23±0.01 (63.16)
Diclo+mefa	0.20±0.01 (61.54)	0.32±0.01 (47.54)	0.21±0.01 (67.69)
Diclo+piroxi	0.19±0.01 (63.46)	0.29±0.02 (52.46)	0.20±0.02 (69.23)

Values are mean ±SEM, n=5, p<0.05vs control

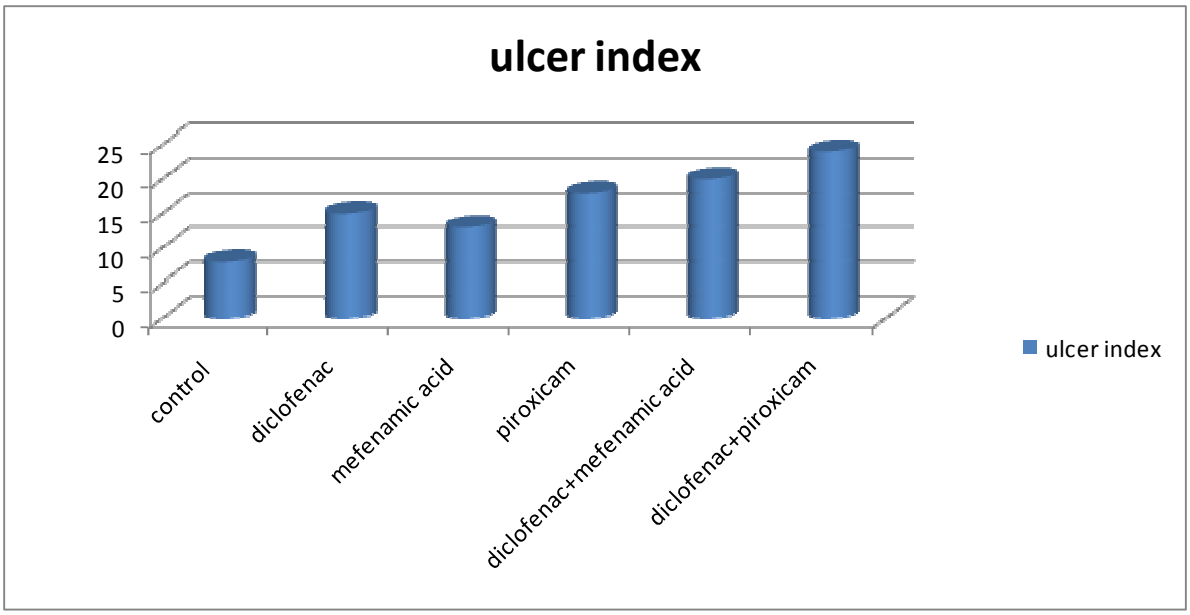

Figure.1. Ulcer index of the various NSAIDs treatments.