Effect of lansoprazole on acute and sub-acute models of inflammation in male Wistar rats: an experimental study

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Abstract

Aims & Objective: To evaluate effect of lansoprazole on acute and sub-acute models of inflammation in rats.

Materials and Methods: The study was carried out in two models of inflammation viz; carrageenan induced rat paw edema and foreign body induced granuloma. Animals were divided into three groups. Control group received 0.5ml of 1% gum acacia suspension while test groups received lansoprazole and aspirin. The rat paw volume was measured with the help of a mercury plethysmograph at regular intervals and percentage inhibition of edema was calculated. In sub-acute model of inflammation, two sterile cotton pellets and two sterile grass piths were implanted subcutaneously in the axilla and groin. The treatment was started on the day of implantation and was repeated every twenty-four hours, regularly, for ten days. On eleventh day, rats were sacrificed and mean granuloma dry weight of cotton pellets for various groups was calculated. The sections of grass piths were sent for histopathological studies.

Results: In the present study, lansoprazole showed significant anti-inflammatory effect in acute as well as sub-acute models of inflammation when compared to control.

Conclusion: Lansoprazole showed anti-inflammatory effect in the current study.

Key words: Lansoprazole, aspirin, inflammation, Carrageenan, PPI, foreign body granuloma.

Introduction

The ability of organisms to get rid of damaged or necrotic tissues is essential for their survival. Host response to noxious stimuli in the form of inflammation accomplishes these goals. Inflammation is a complex reaction in tissues that consists mainly of vascular and cellular response. The inflammatory response is closely intertwined with the process of repair. During repair, the injured tissue is replaced through regeneration of native parenchymal cells or by filling of the defect with fibrous tissue (scarring) or most commonly by a combination of these two processes.1

Many steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are in clinical use to reduce inflammation. Also, some other drugs like penicillamine,2 allopurinol etc.,2 have been in clinical use to treat inflammatory conditions like rheumatoid arthritis, gout etc. Some adrenergic agonists,3 calcium channel blockers4, calcium5, angiotensin receptor blockers6,7, dipeptidyl peptidase 4 inhibitors8, statins9,10,11 and sulfamethizole12 have also been reported to possess anti-inflammatory activity in experimental studies though they are not routinely used in the treatment of inflammatory disorders. But, these drugs are not completely devoid of adverse effects.2 Hence the search for safer and better anti-inflammatory agents continues.

Gastro-oesophageal reflux disease (GERD) and peptic ulcer disease (PUD) are among the most prevalent gastro-intestinal inflammatory disorders. Population based studies show that up to 15% of individuals have heartburn or regurgitation at least once a week and 7% have daily.13 Untreated GERD may cause erosive or non-erosive oesophagitis. Erosive oesophagitis may lead to peptic stricture due to fibrosis or intestinal metaplasia (Barrett’s oesophagus) which is risk factor for oesophageal adenocarcinoma. Reflux may also cause peptic ulcer formation in oesophagus.

Histopathological features of GERD and peptic ulcer show neutrophils with other inflammatory infiltrates and granulation tis-
These inflammatory cells can cause epithelial cell injury by production of reactive oxygen or nitrogen species. Also, inflammatory response influences the clinical outcome of PUD such as progression to pangastritis and multifocal atrophic gastritis, gastric cancer etc. Other PUD related complications are gastrointestinal bleeding, perforation and gastric outlet obstruction secondary to inflammation and scar formation.

Interestingly, proton pump inhibitors (PPIs) which are routinely used in treatment of GERD and PUD are reported to possess anti-inflammatory properties, in vitro. Various in vitro studies have shown that lansoprazole suppresses induction of inflammatory mediators like TNF-α, IL-1α, IL-6 and induce protective enzyme. PPIs have also been reported to inhibit certain neutrophil functions, like Reactive Oxygen Species (ROS) release, chemotaxis and neutrophil-endothelial cell interactions of which contribute to the development and progression of inflammation. In addition, lansoprazole has been found to ameliorate ischemic reperfusion induced intestinal mucosal damage in experimental rats.

However, review of literature indicates scarcity of animal studies regarding the effects of lansoprazole and other PPIs on inflammation. In view of role of inflammation in GERD and PUD and paucity of information regarding effects of PPIs on inflammation, the present study was planned to evaluate the effect of lansoprazole on acute and sub-acute models of inflammation.

**Materials & Methods**

Adult male healthy Wistar rats weighing 175 ± 25 g were obtained from the central animal house of institution and were acclimatized to 12:12 h light - dark cycle for 10 days prior to the day of experimentation. They were maintained on standard rat chow pellet and water ad libitum. The study was approved by the Institutional Animal Ethics Committee (IAEC). (Ref: MDC/PG/224; dated 08-10-2010)

Rats were divided into three groups of six each (n=6). They were starved overnight with water ad libitum prior to the day of experiment. Group I - Control group received 0.5ml of 1% gum acacia suspension orally, Group II – Standard group received aspirin in the dose of 200 mg/kg body weight equivalent to 2222 mg of clinical dose orally. Group III – Test group received lansoprazole in the dose of 16.2 mg/kg body weight equivalent to 100 gm body weight. Aspirin was administered thirty minutes prior, while lansoprazole was administered one hour prior to the induction of edema for acute inflammation study.

### 1. Carrageenan induced rat paw edema

0.05ml of 1% carrageenan in normal saline was injected into the sub-plantar region of one of the hind paws. A mark was put on the hind limb at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume in millilitre was measured with the help of mercury plethysmograph by mercury displacement method at zero hour i.e. immediately after injecting carrageenan. The same procedure was repeated at 0.5, 1, 3, 4 and 5 hours. The difference between 0 hour and subsequent reading was taken as actual edema volume. The percentage inhibition of edema in the various treated groups was then calculated by using the formula,

$$\text{Percentage Inhibition of edema} = \frac{1 - \frac{\text{Mean increase in paw volume in control group}}{\text{Mean increase in paw volume in treated group}}} \times 100$$

### 2. Foreign Body Induced Granuloma Method

After clipping the hair in axillae and groin, under thiopentone anaesthesia, two sterile cotton pellets weighing 10mg and two sterile grass piths (25x2mm) were implanted, subcutaneously, through a small incision. Wounds were then sutured and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the experiment. The treatment was started on the day of implantation and was repeated every twenty-four hours, regularly, for ten days.

On eleventh day, the rats were sacrificed with an overdose of anaesthesia to remove the cotton pellets and grass piths. The grass piths were sent to pathologist for histopathological studies. The pellets, free from extraneous tissue were dried overnight at 60°C to note their dry weight. Net granuloma formation was calculated by subtracting initial weight of cotton pellet (10mg) from the weights noted. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 gm body weight. The percentage inhibition of granuloma dry weight was calculated using the formula,

$$\text{Percentage Inhibition of granuloma dry weight} = \frac{\text{Dry weight of granuloma in control group} - \text{Dry weight of granuloma in treated group}}{\text{Dry weight of granuloma in control group}} \times 100$$

### Statistical analysis:

The data was analyzed by one way ANOVA followed by post hoc Dunnett’s test. ANOVA followed by Bonferroni’s test was used to compare aspirin and lansoprazole group. Statistical analysis was done using Graph pad prism software and p < 0.05 was considered statistically significant.

### Result

In the present study lansoprazole was investigated for its possible anti-inflammatory effect, in acute and sub-acute models of inflammation.

### Acute inflammation (Carrageenan induced paw edema):

The mean paw edema volumes in millilitres (ml), as measured by mercury displacement using a plethysmograph, for control, aspirin treated, and lansoprazole treated groups at various time intervals, and calculated percentage inhibitions of aspirin and lansoprazole treated groups are tabulated in Table 1 and graphically represented in Figure 1 and 2.

Aspirin treated group showed statistically significant inhibition of paw edema volume when compared to control. Also, Inhibition of paw edema volume in lansoprazole treatment group was
statistically significant, when compared to control which clearly show the anti-inflammatory effect of lansoprazole in acute model of inflammation. Further anti-inflammatory effect of lansoprazole was compared with anti-inflammatory effect of aspirin. It was found that anti-inflammatory effect of lansoprazole was inferior to aspirin. (Table-1).

**Sub-acute inflammation (foreign body induced granuloma):**

The mean dry weight of ten-day old granuloma, expressed as mg percent (mg/100 g) body weight of rat, in control group was 43.57±0.83. In aspirin treated group, it was significantly decreased with the mean value of 26.55±0.74 and percentage inhibition of 39.06%. Similarly, lansoprazole treated group exhibited statistically significant decrease in granuloma dry weight with mean value of 28.73 ± 0.48 with percentage inhibition of 30.46% when compared to control. (Table-2, Figure 3 and 4)

Further, mean granuloma dry weight of lansoprazole was compared with mean granuloma dry weight of aspirin group and it is found that there was no statistically significant difference (p>0.05). It shows that the anti-inflammatory effect of lansoprazole was comparable to aspirin in sub-acute model of inflammation (Table-2 and Figure 4).

These results were further confirmed by histopathological studies. The tissue was processed in pathology department and sections of granulation tissues were stained with haematoxylin and eosin stain. Histopathological study showed there is increase in number of fibroblasts, thick fibrous tissue and excessive granulation tissue in the control group. Whereas in aspirin and lansoprazole treated groups it is found that number of fibroblasts were decreased, scanty collagen tissue and decrease in thickness of fibrous tissue (Figure 5a, 5b, 5c).

### Table 1: Effect of control, aspirin and lansoprazole treatments on carrageenan induced paw edema.

<table>
<thead>
<tr>
<th>Time after carrageenan injection</th>
<th>Control</th>
<th>Aspirin</th>
<th>Lansoprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paw edema In ml (Mean±SEM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>½ hr</td>
<td>0.32 ± 0.01</td>
<td>0.15± 0.01**</td>
<td>0.28± 0.01** , öö</td>
</tr>
<tr>
<td>1hr</td>
<td>0.51 ± 0.01</td>
<td>0.26±0.01**</td>
<td>0.46± 0.01**, öö</td>
</tr>
<tr>
<td>3hr</td>
<td>0.84 ± 0.01</td>
<td>0.36±0.02**</td>
<td>0.65± 0.02**, öö</td>
</tr>
<tr>
<td>4hr</td>
<td>0.99 ± 0.02</td>
<td>0.43±0.02**</td>
<td>0.73± 0.02**, öö</td>
</tr>
<tr>
<td>5hr</td>
<td>0.86 ± 0.02</td>
<td>0.32±0.01**</td>
<td>0.69± 0.02**, öö</td>
</tr>
</tbody>
</table>

ANOVA: p<0.0001
Post hoc analysis: By Dunnet’s Test: **P<0.01, By Bonferroni’s test: öö P <0.01

### Table 2: Effect of control, aspirin and lansoprazole treatments on granuloma dry weight.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug Treatment(Oral)</th>
<th>Mean granuloma dry weight mg/100gm body weight (Mean ± SEM)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>43.57 ± 0.83</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Aspirin</td>
<td>26.55 ± 0.74**</td>
<td>39.06</td>
</tr>
<tr>
<td>3</td>
<td>Lansoprazole</td>
<td>28.73 ± 0.48**</td>
<td>30.46</td>
</tr>
</tbody>
</table>

ANOVA: p<0.0001
Post hoc analysis: By Dunnet’s Test: ” p<0.01, By Bonferroni’s Test: p>0.05

**Figure 1: Effect of control, aspirin and lansoprazole treatments on carrageenan induced paw edema.**
Discussion

As mentioned in the introduction, the present study was planned to evaluate the effect of lansoprazole in acute and sub-acute models of inflammation in male Wistar rats.

Results of the present study clearly indicate that lansoprazole showed significant anti-inflammatory effect in acute as well as sub-acute models of inflammation when compared to control group. Anti-inflammatory effect of lansoprazole was inferior to aspirin in acute model of inflammation, but in sub-acute model of inflammation it was found to have anti-inflammatory effect comparable to aspirin. PPIs, being weak bases, can be expected to accumulate in the acidic environment at the site of inflammation. Accumulation and activation of PPIs in the acidic environment of inflammation after repeated dosing could be the explanation for comparable efficacy with aspirin in sub-acute model of inflammation.

Recent in vitro studies have suggested a number of mechanisms whereby PPIs can exert anti-inflammatory effects. Observations of the current study are in agreement with the earlier study reports stating that proton pump inhibitors may have anti-inflammatory activity. The PPIs are weak bases which accumulate in the acid secreting mucosa and block the p-type H⁺K⁺ATPase of gastric parietal cells that secrete acid into the gastric lumen. Interestingly, in addition to gastric parietal cells, some non-gastric cells like neutrophils and vascular endothelial cells have been reported to have vacuolar (v-type) H⁺ATPases. Vacuolar proton pumps have been found prominently in the membranes of the phagolysosomes of neutrophils. When neutrophils are activated by chemotactic factors, these vacuolar H⁺ATPases pump H⁺ into the phagolysosome. This lysosomal acidification appears to play a role in mediating the neutrophil’s oxidative burst and the rapid release of toxic ROS. Some of these v-type H⁺ATPases have been found to be inhibited, in vitro, by PPIs. In the process of inflammation, neutrophil-endothelial cell interactions mediated by adhesion molecules are important for transmigration of neutrophils through the endothelium. In an in vitro study in human peripheral neutrophils and umbilical vein endothelial cells, lansoprazole has been found to attenuate the expression of adhesion molecules like CD11b and CD18 on neutrophils and intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) on endothelial cells. PPIs have also been reported to inhibit chemotaxis of neutrophils in studies on human polymorphonuclear leucocyte (PMN) suspensions. Therefore it is conceivable that treatment with PPIs might interfere with neutrophil functions, like ROS release, adhesion molecule expression and chemotaxis, all of which contribute to the development and progression of inflammation. Lansoprazole has been shown to decrease the levels of various pro-inflammatory cytokines including IL-6 and TNF-α in cultured human neuroblastoma cells. Furthermore, in a human monocytic cell line, lansoprazole has been found to suppress the production of TNF-α and IL-1α induced by lipopolysaccharide and H. pylori.
water-soluble extract. It does so by inhibition of nuclear factor (NF)-κB and extracellular signal regulated kinase (ERK) as well as by prevention of phosphorylation and degradation of inhibitory factor-κB. These intracellular signaling pathways from receptors to the nucleus, which activate the production of inflammatory cytokines and proteins, could be the therapeutic targets of PPIs for prevention and regulation of deleterious inflammatory responses. Heme oxygenase (HO) is an enzyme involved in heme catabolism, a process in which the oxidation of heme leads to the production of iron, biliverdin and carbon monoxide. Two mammalian HO isoforms have been identified, HO-1 (inducible) and HO-2 (constitutive). HO-1 is a stress-responsive protein. HO-1 is inducible by a vast array of stimuli, including oxidative stress, ultraviolet radiation, ischemia-reperfusion, heavy metals, bacterial lipopolysaccharide (LPS), cytokines, nitric oxide, and substrate heme. HO-1 is a potent anti-inflammatory mediator and induction of HO-1 inhibits inflammation. Lansoprazole has been found to induce HO-1, *in vitro*, in rat gastric epithelial cells. Lansoprazole has also been found to protect against ischemia-reperfusion injury of the bowel in rats. Beneficial effects of PPIs have been reported in the patients with inflammatory bowel disease. Anti-inflammatory effects of the PPIs might influence a variety of inflammatory disorders, both peptic and non-peptic, within and outside of the gastrointestinal tract.

Since lansoprazole showed significant anti-inflammatory activity, their use can be promoted in treating GERD, esophagitis, Barrett’s esophagus, gastritis including PUD and complications like peptic strictures, gastric outlet obstruction. In the treatment of acid peptic disorders, lansoprazole can be expected to have dual benefit by virtue of its acid lowering as well as anti-inflammatory effect. This study also suggests that lansoprazole might have beneficial effects in number of inflammatory diseases, in which acid and pepsin have no role like non reflux inflammatory conditions (e.g. Eosinophilic esophagitis), Ulcerative colitis etc. For patients with upper gastrointestinal symptoms of uncertain etiology, such as non-ulcer dyspepsia, improvement with PPI therapy is considered as *prima facie* evidence for acid-peptic disease. Anti inflammatory effect of lansoprazole may pose a serious challenge to the common clinical practice of assuming that a symptomatic response to PPI treatment is proof of an underlying acid peptic disorder.

However, these speculations need to be confirmed clinically. Clearly, the role of PPIs as anti-inflammatory agents is an area that warrants further investigation.

**Conclusion**

In the current study, lansoprazole have shown significant anti-inflammatory activity in acute and sub-acute models of inflammation. In acute model of inflammation, lansoprazole shown significant anti-inflammatory effect when compared to control, but it was inferior to aspirin. In sub-acute model of inflammation, lansoprazole have shown comparable anti-inflammatory effect with aspirin. The findings of the present experimental study appear to be clinically relevant. Use of PPIs in treating GERD and PUD can reduce the inflammatory complications, by virtue of their anti-inflammatory activity, in addition to acid lowering property. Also, PPIs might have a role to play in the treatment of variety of inflammatory disorders, both peptic and non-peptic, within and outside of the gastrointestinal tract.

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**Conflict of interest**

No conflict of interest

**References**


