ABSTRACT

**Background & objectives:** NSAIDS induced gastric ulcer is very common nowadays; a number of drugs are used to prevent NSAIDS used ulcer. In this study we evaluated the antiulcerogenic effect i.e prevention of Aspirin induced ulcer and antisecretory effect of Roxatidine, the second generation H2 receptor antagonist and one of the widely used proton pump inhibitor Rabeprazole in wistar rats.

**Methods:** The antisecretory effect of both drug and control is compared by pyloric ligation method. Free and total titrable acid is measured and compared. The antiulcerogenic effect is compared by aspirin (200mg/kg) induced gastric ulcer in wistar rats. Ulcer index of both the drug and control is determined and compared. **Result:** In our study, Rabeprazole (20 mg/kg) was more effective than roxatidine (5mg/kg) in reducing free and total titrable acids and in reduction of ulcer (p<0.05). Both the drugs were superior to that of control (p<0.001). **Interpretation & conclusion:** Based on our findings, we presume that the anti-secretary and antiulcerogenic (i.e Prevention of NSAIDS induced gastric ulcer) properties of Rabeprazole is better than that of roxatidine in animal model.

**KEYWORDS:** Aspirin; NSAIDS, Rabeprazole; Roxatidine, Ulcer Index.
INTRODUCTION

The relationship between nonsteroidal anti-inflammatory drugs (NSAIDs) and gastroduodenal injury is well established.\cite{1} Many factors such as gastric acid, pepsin secretion, gastric microcirculation, prostaglandin E2 (PGE2) content, pro-inflammatory cytokines interleukin(IL)-1 and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), contribute to the formation of gastric mucosal damage and subsequently to ulcer development \cite{2-4}. Among the NSAIDs, Aspirin and diclofenac are commonly associated with peptic ulcer disease.\cite{5} Multiple agents, including antacids, H2-receptor antagonists, and proton pump inhibitors, are currently available for the treatment of gastric ulcers.\cite{6} Proton pump inhibitors are the most potent inhibitors of gastric acid secretion.\cite{7} H2-receptor antagonists are other alternative to Proton pump inhibitors and in some studies the H2 receptor antagonists have demonstrated comparable efficacy to them.\cite{8, 9} Roxatidine is the Second Generation H2-Receptor antagonist has longer duration of action with greater 24 hour acid suppression.\cite{10} Rabeprazole is commonly used Proton Pump Inhibitor. Although, both the drugs have been frequently used in various types of peptic ulcer diseases for quite some time, a head to head comparison is still wanting. Therefore, we went ahead to take up this issue to compare the anti-gastric acid secretory and anti-ulcerogenic effect of roxatidine and rabeprazole by using aspirin induced gastric ulcer model of rat. This study can also serve as a platform for the future clinical studies.

MATERIAL AND METHODS

All the experiments were approved by the Institutional Animal Ethics Committee for conducting animal experiments.

Chemicals

Roxatidine 5mg/kg body weight was used as experimental drug while Rabeprazole 20mg/kg body weight acted as active comparator. Aspirin 200mg/kg body weight was used as ulcer inducing agent using gum acacia as vehicle. Suspensions of the individual drugs were made before starting experiment, and kept in washed, dried bottles.

Experimental animals

Adult wistarWister rat of either sex weighed between 180-200 gm was housed separately. The animals were left for 48 hrs to acclimatize to the animal room conditions. They were maintained in standard laboratory conditions of temperature 22±2°C, humidity, 12 hours light and dark cycles fed with standard pellet diet and tap water ad libitum.
Pyloric Ligated Gastric Secretion
A total of 18 Wistar wister rats were randomly divided into three groups (n = 6) and were fasted for 36-48 hours with free access to water. One hour after oral administration of single dose of vehicle (1ml saline) as control, roxatidine (5mg/kg) as experimental drug and rabeprazole(20 mg/kg) as active comparator, the pylorus of each rat was tied under urethane anesthesia (1.25 gm/kg of body weight I.M ) and abdomen incision were closed. Six hours later, the animals were sacrificed, the abdomen opened and another ligature placed around the esophagus close to the diaphragm. The stomach was removed and the gastric juice produced by each was collected.

Free Acidity and Total Acidity
The gastric contents were centrifuged at 2000 rpm for 10 minutes. 1 ml of supernatant fluid was taken with a pipette and diluted it to 10 ml with distilled water. PH of the solution is determined with PH meter. The solution is titrated against 0.01N NaOH using topfers reagent as an indicator to the end point till the solution turns to orange colour. The volume of NaOH needed corresponds to free acidity. Then three drops of phenolphthalein was added and titrated till definite red tinge appeared. The total volume of NaOH required, corresponds to the total acidity. Acidity mEq/L/100gm can be expressed as
\[
\text{Acidity} = \frac{(\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100)}{0.1}
\]

Aspirin Induced Gastric Ulcer Method
Animals were kept fasting for 24 hours before experiment. One hour before experiment the control group of the animals were given 2 ml of normal saline. To the experimental group of animals Rabeprazole was given 20 mg/kg of body weight orally Roxatidine was given in 5 mg/kg of body weight orally with the help of fine rubber catheter. Half an hour after this both groups of animals received aspirin power suspended in 1% gum acacia in doses of 200 mg/kg body weight. It was introduced in stomach through a fine rubber catheter. Neither food nor water was allowed during this period. Six hours after aspirin administration wistar rats were sacrificed with over dose of ether anaesthesia. Abdomen was opened with midline incision. Stomach was freed of mesentery and removed. It was cut along the greater curvature and washed under direct stream of cold water. Lesions were examined by necked eye. Ulcer in each wistar rats was judged to be positive or negative on the basis of presence of absence of one or more haemorrhagic area.
Calculation of Ulcer Index
Ulcer index was then calculated from the glandular portion of the stomach by using a handheld magnifying lens and a measuring tape.

Calculations:-

Ulcer index is calculated as per R.K. Goyal.[11,12]

Ulcer Index = 10/X

Where X = Total mucosal surface ÷ Total ulcerated area

Measuring protocols

- Each lesion was measured along its greatest length
- Five petechiae, where present were considered equivalent to an area of 1sqmm
- Total glandular area and total ulcerated mucosa were measured for calculation of the ulcer index

RESULTS

In the present study effects of roxatidine the second generation H2 receptor antagonist is compared with rabeprazole, one of the commonly used proton pump inhibitor with a view to find out these agents, offered any protection against aspirin induced ulceration and their effects on total and titrable acidity in pylorous ligation method. Both the drugs in tested doses produced a decrease in ulcer index as compared to the control. Both the drugs i.e. roxatidine and rabeprazole revealed to have protection against aspirin (200 mg/kg body weight) induced ulcers in rats as well as decrease the output of total and titrable acids in comparison to control. Maximum protection was seen in the rabeprazole treated group. The difference is statistically significant both in prevention of aspirin induced gastric ulcer and antisecretory effect.

The statistical analysis was performed using the software Graphpad prism version 6.
Table 1: Effect of roxatidine (5mg/kg of body weight) rabeprazole (20 mg/kg of body weight) orally on gastric acid secretion in six hours pylorus ligated wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Volume of Gastric Juice In ml Mean ± SEM</th>
<th>TITRABLE ACIDITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Free acid ml 0.1N HCl/100 ml of gastric Juice</td>
</tr>
<tr>
<td>Control (2 ml Normal Saline)</td>
<td>6.40 ± 0.08</td>
<td>46.48 ± 0.57</td>
</tr>
<tr>
<td>Roxatidine (5 mg/kg body weight)</td>
<td>3.19 ± 0.05***</td>
<td>17.23 ± 0.09***</td>
</tr>
<tr>
<td>Rabeprazole (20 mg/kg body weight)</td>
<td>1.9 ± 0.09***</td>
<td>9.97 ± 0.27***</td>
</tr>
<tr>
<td>P value</td>
<td>***&lt;0.001 in comparison to control &lt;0.05 rabeprazole versus roxatidine</td>
<td>***&lt;0.001 in comparison to control &lt;0.05 rabeprazole versus roxatidine</td>
</tr>
</tbody>
</table>

P-value obtained by using ANOVA followed by Kruskal-Wallis test as post-hoc test

Table 2: Effect of roxatidine (5mg/kg of body weight) rabeprazole (20 mg/kg of body weight) orally on aspirin induced gastric ulcer in wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage of Wistar Rats ulcerated</th>
<th>Ulcer index (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin (200 mg/kg body wt)</td>
<td>100 %</td>
<td>9.57 ± 0.53</td>
</tr>
<tr>
<td>Roxatidine (5 mg/kg body weight)</td>
<td>33.33 %***</td>
<td>4.65 ± 0.24***</td>
</tr>
<tr>
<td>Rabeprazole (20 mg/kg body weight)</td>
<td>16.67 %***</td>
<td>2.63 ± 0.32***</td>
</tr>
<tr>
<td>P value</td>
<td>***&lt;0.001 in comparison to control &lt;0.05 rabeprazole versus roxatidine</td>
<td>***&lt;0.001 in comparison to control &lt;0.05 rabeprazole versus roxatidine</td>
</tr>
</tbody>
</table>

P-value obtained by using ANOVA followed by Kruskal-Wallis test as post-hoc test

**DISCUSSION**

NSAIDs have been shown to cause epigastric distress, heartburn, gastric ulceration and haemorrhage in experimental animal and in human being. Aspirin and diclofenac are frequently found to be responsible for the development of peptic ulcer disease.[5] Even low dose aspirin has the potential to cause peptic ulcer in 10-40% patients and increases the risk of upper GI bleeding by two fold.[13] The prevalence of peptic ulcer is around 20% among the non-aspirin users; however the risk of upper GI bleeding is 4-6 times more in them.[13] The use of NSAIDs is common in elderly population.[14] This is consistent with previous studies and reflects that no dose is safe from gastrointestinal toxicity.[15, 16] Proton pump inhibitors and H2 receptor blockers are the two frontline drugs used in prevention and treatment of NSAIDs induced gastric injury or peptic ulcers. Among the Proton pump inhibitors the
percent protection in ulcer index is maximum for rabeprazole than the other proton pump inhibitors (viz Omeprazole and Lansoprazole).\textsuperscript{17, 18} Roxatidine has longer duration of action and amongst the newer H2 receptor blocker. Although H2-receptor antagonists are other alternative to Proton pump inhibitors and it is reflected in some clinical study that they have comparable efficacy in acid peptic disorder,\textsuperscript{8, 9} our study demonstrated rabeprazole to be more effective in prevention of aspirin induced gastric ulcer in animal model and it has better antisecretory action than a second generation H2 receptor antagonist roxatidine. Rabeprazole, being a direct inhibitor of the H\textsuperscript{+}-K\textsuperscript{+}-ATPase pump (Proton pump) present on parietal cells of the gastric mucosa, causes a more profound and prolonged acid suppression.\textsuperscript{17, 18} Roxatidine, on the other hand, is a competitive antagonist of histamine at the H2 receptor, which in turn stimulates the proton pump to secrete acid. Since NSAIDS induced gastric injury is very common health related problem now a days, and with increased incidence of musculoskeletal disorder and ischaemic heart disease, where low dose aspirin is of paramount importance, these agents play a crucial role in the prevention of gastric and duodenal ulcer formation.

However, this is a pre-clinical experiment. In future, more detailed study involving human subjects can provide us further insight about these agents.

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Conflict of interest: None to the best of our knowledge.

REFERENCES