EFFECTS OF A PROTON PUMP INHIBITOR OMEPRAZOLE AND THE H₂-RECEPTOR BLOCKER RANITIDINE ON HEALING OF STRESS-INDUCED ULCERS IN RATS

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ABSTRACT
A study was designed to compare the effect of omeprazole (orally) as a proton pump inhibitor and ranitidine (orally & i.m.) as H₂-receptor antagonist on stress-induced gastric ulcer in male albino rats (the mean ulcer score, and ulcer and preventive indices) and the effect of these drugs on gastric secretion (the mean volume of gastric juice, acid concentration, acid output and pepsin and mucous concentration). The incidence of ulceration was lowest in rats pretreated with omeprazole and ranitidine in doses of 5.4 mg/kg b.wt (orally) and 10 mg/kg b.wt (i.m.) respectively. Pretreatment of rats with omeprazole or ranitidine (the same dose) produced the lowest ulcer score and ulcer index and the highest preventive index. The mean volume of gastric contents (collected for 4 hrs) was lowest in animals pretreated with a dose of 5.4 followed by 3.6 then 1.8 mg/kg b.wt omeprazole respectively. It was found that the greatest reduction of the mean acid concentration, acid output and pepsin concentration was evident with omeprazole in a dose dependent manner. It was found also that omeprazole significantly increases the mucus content of gastric secretion while ranitidine significantly decrease it.

INTRODUCTION
Certainly, ranitidine is a highly selective H₂-receptor antagonist. It is known to inhibit gastric secretion elicited by muscarinic agonists and histamine or other H₂-agonists in a dose dependent manner and it can protect experimental animals from induced gastric ulceration (4).

Obviously, ranitidine is absorbed from gastro-intestinal tract with a peak plasma concentration reaching after about 2 hours, the elimination half-life from plasma is around 2-3 hours (weakly bound to plasma proteins). A small proportion of ranitidine is metabolized in liver to N-oxide, S-oxide and desmethyl ranitidine. Approximately 30% of on oral dose and 70% of i.v. dose is excreted unchanged in urine in 24 hours (2).

Also, omeprazole (a known inhibitor of acid secretion) blocks the action of H⁺/K⁺- ATPase, (the proton pump) the final step in the synthesis and release of gastric acid (3).

As previously known, omeprazole is rapidly absorbed after oral administration reaching the peak plasma level within one hour of dosing, the elimination half life is an hour and the duration of the effect 3-4 days after a single dose. It is highly bound (about 95%) to plasma proteins (4). Omeprazole is excreted in urine after its metabolism in the liver. Omeprazole is highly effective in healing ulcer, rapid symptoms relief and far superior to the H₂-receptor antagonist ranitidine in preventing ulcer recurrence (5).

MATERIAL AND METHODS
Drugs :
1- Omeprazole (Epirazole®) (Eipico, Egypt) 5 methoxy
2- (4- methoxy -3.5 dimethyl-2- pyridyl methyl sulphinyl) benzimidazole.
2- Ranitidine (Zantac) (Glaxo-welcome, England), 1.1 -
-2-nitro - hydrochloride.

Animals :
Two groups of adult albino rats of local strain weighing 150-170 g were employed for this study. The animals were divided into two groups :

Group I : Contains 48 rats divided into eight subgroups . These groups were employed to study the effect of different doses of omeprazole and ranitidine on the incidence and severity of the induced gastric ulcers. Each drug was administered is equal volume by gavage as follows.

Subgroup treatment (single dose) :
1-This group received 1 ml of the vehicle [carboxymethylcellulose 1% suspension, CMS] orally followed by 1 ml of distilled water 10 minutes later and served as control group.
2-Received ranitidine 5 mg/kg b.wt i.m.
3-Received ranitidine 10 mg/Kg b. wt i.m.
4-Received ranitidine 15 mg/kg b.wt orally.
5-Received ranitidine 30 mg/kg b.wt orally.
6-Received omeprazole 1.8 mg/kg b.wt orally.
7-Received omeprazole 3.6 mg/kg b.wt orally.
8-Received omeprazole 5.4 mg/kg b.wt orally.

Group II : consists of 6 subgroups each at six . These groups were used to study the effect of omeprazole and ranitidine on gastric secretion. Each test compound was administered by gavage 10 minutes before immobilization in a single dose followed, 1 hour later, by ligation of the pylorus using the Shay technique (6).

This group was subdivided into 6 subgroups each of 6 animals as follows :

Subgroup treatment (a single dose ) :
1-Received 1 ml of the vehicle (CMS) orally followed by 1 ml of distilled water 10 minutes later and served as control.
2- Received omeprazole 1.8 mg/kg b.wt orally.
3- Received omeprazole 3.6 mg/kg b.wt orally.
4-Received omeprazole 5.4 mg/kg b.wt orally.
5-Received ranitidine 15 mg/kg b.wt orally.
6-Received ranitidine 30 mg/kg b.wt orally.

Gastric ulceration was induced as described before (7). The number and severity of discrete areas of damage in the glandular mcosa were calculated according to previously reported method (8). Stomach ulceration was expressed in term of ulcer index UI (UI = mean ulcer score of group of animal similarly treated X% of ulcerated animals of the group) (9).
Subgroup treatment (a single dose):
1. Received 1 ml of the vehicle (CMC) orally followed by 1 ml of distilled water 10 minutes later and served as control.
2. Received omeprazole 1.8 mg/kg b.wt orally.
3. Received omeprazole 3.6 mg/kg b.wt orally.
4. Received omeprazole 5.4 mg/kg b.wt orally.
5. Received ranitidine 15 mg/kg b.wt orally.
6. Received ranitidine 30 mg/kg b.wt orally.

Gastric ulceration was induced as described before. The number and severity of discrete areas of damage in the glandular mucosa were calculated according to previously reported method. Stomach ulceration was expressed in term of ulcer index UI (UI = mean ulcer score of group of animal similarly treated X% of ulcerated animals of the group).

The preventive effect of any of the antiulcer agents used was calculated according to reported method.

Preventive index (PI) = \( \frac{UI (control) - UI (treated)}{UI (control)} \times 100 \)

**Effect of the test drug on gastric secretion:**

The effect of drugs on gastric secretion was tested by Shay rats (Pylorus ligated) technique and collection of all gastric juice that accumulate during a given time interval. After 24 hours fasting, animals received orally either the vehicle or the anti ulcer drugs. One hour later the pylorus was ligated under ether anaesthesia. Animals were killed 4 hours after pylorus ligation. The gastric juice was taken, measured and centrifuged, the supernatant fluid was analysed for titratable acidity, proteolytic acidity of pepsin and mucous concentration.

**RESULTS AND DISCUSSION**

Rats are susceptible to stress induced gastric ulceration which is histologically similar to human stress ulcer. This may be due to the absence of gall bladder, in rats, which result in continuous entrance of bile (strong gastric secretagogue) into the intestine. In the present study, the incidence of stress ulceration in rats was 100%, ulcer score was 3.2 and the ulcer index was 320, these results are in accordance with that reported before.

The probable mechanism of stress ulcer may be the increase of gastric motility by stress resulting in mechanical rubbing of gastric mucosa. Furthermore, the increase of acidity may be associated with vagal over-activity and blocking of the ischaemic effect protects against the gastric stress ulceration. The release of thyrotropin releasing hormone from the stomach wall (which is mediated by both muscarinergic and histaminergic H₂ system) is important in the pathogenesis of stress ulcer.

**Effect of ranitidine and omeprazole on stress induced gastric ulceration:**

Ranitidine decrease the incidence of gastric ulceration and significantly decrease the mean ulcer score and ulcer index in male albino rats (15.3 mg/kg b.wt orally and 5-10 mg/kg b.wt i.m.).

The drug significantly increase the preventive index against stress ulceration (Table 3). It was found also that omeprazole (1.8, 5.4 mg/kg orally) decrease significantly the incidence of gastric ulceration, the mean ulcer score and ulcer index. It also increased significantly the preventive index and produced a high protective effect against gastric ulceration. These results are in accordance with that reported before.

**Effect of omeprazole on gastric secretions of pylorus ligated rats:**

In this study, omeprazole (dose dependant manner) significantly decreased the gastric volume as well as acid concentration (um Eg/l), acid output and the proteolytic activity of pepsin (Table 4). These results are in accordance with that reported before. On the other hand omeprazole (dose dependantly) significantly increased the glycoprotein content of gastric juice (table 4), a result which was compatible with that obtained before, who suggested that the enhancement of gastric mucosa secretion contributes to this protective action.

**Effect of Ranitidine on gastric secretion of pylorus ligated rats:**

It has been found that ranitidine (15 & 30 mg/kg orally) significantly decreased the acid concentration, acid output, proteolytic activity of pepsin (Table 4). These results were in the same direction with that obtained before.

It has been reported that the output of pepsin which is secreted by the chief cells of gastric gland (mainly under cholinergic control), generally fall in parallel with the reduction in gastric juice volume.

Ranitidine significantly decreased the mucous concentration in gastric juice (Table 4) and this agrees with the results obtained before. Finally, it is advised that patient treated with omeprazole (60 mg orally) or ranitidine (100 mg i.m.) to administer the drug one hour before induction of anaesthesia to avoid incidence of stress ulcer.
Table (2): Optical density of various pepsin concentrations at a wave length 280 nm.

<table>
<thead>
<tr>
<th>Pepsin concentration (mg/ml)</th>
<th>Mean optical density ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.13 ± 0.003</td>
</tr>
<tr>
<td>0.3</td>
<td>0.12 ± 0.004</td>
</tr>
<tr>
<td>0.5</td>
<td>0.22 ± 0.004</td>
</tr>
<tr>
<td>0.4</td>
<td>0.27 ± 0.021</td>
</tr>
<tr>
<td>0.6</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>0.8</td>
<td>0.35 ± 0.002</td>
</tr>
<tr>
<td>0.7</td>
<td>0.36 ± 0.01</td>
</tr>
</tbody>
</table>

* Mean of 3 experiments for each concentration.

Fig. (1): Standard calibration curve showing the mean optical density of various pepsin concentrations at 280nm.
* Vertical lines represent the standard deviation of the mean.

Table (2): Optical density of various hexose concentrations at a wave length 500 nm.

<table>
<thead>
<tr>
<th>Hexose concentration (mg/ml)</th>
<th>Mean optical density ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.01 ± 0.000</td>
</tr>
<tr>
<td>0.2</td>
<td>0.1 ± 0.022</td>
</tr>
<tr>
<td>0.4</td>
<td>0.25 ± 0.039</td>
</tr>
<tr>
<td>0.6</td>
<td>0.4 ± 0.028</td>
</tr>
<tr>
<td>0.7</td>
<td>0.48 ± 0.025</td>
</tr>
</tbody>
</table>

* Mean of 3 experiments for each concentration.

Fig. (2): Standard calibration curve showing the mean optical density of various hexose concentrations at 500nm.
* Vertical lines represent the standard deviation of the mean.
Table (3) : Effect of ranitidine and omeprazole on the incidence of gastric ulceration, mean ulcer score, ulcer index and preventive index in immobilized rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Omeprazole</th>
<th>Ranitidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.8 mg/kg orally</td>
<td>3.6 mg/kg orally</td>
</tr>
<tr>
<td>Incidence of gastric ulceration</td>
<td>100%</td>
<td>60%</td>
<td>60%</td>
</tr>
<tr>
<td>Mean ulcer score</td>
<td>3.2±0.07</td>
<td>1.0±0.035***</td>
<td>0.8±0.13***</td>
</tr>
<tr>
<td>Ulcer index</td>
<td>320</td>
<td>60</td>
<td>48</td>
</tr>
<tr>
<td>Preventive index</td>
<td>-</td>
<td>81.25%</td>
<td>85%</td>
</tr>
</tbody>
</table>

*** Significant at P < 0.001

Table (4) : Effect of ranitidine and omeprazole given orally on basal gastric secretions (colected for 4 hours) of male albino rats (means ± SE) n = 4.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Omeprazole</th>
<th>Ranitidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.8 mg/kg orally</td>
<td>3.6 mg/kg orally</td>
</tr>
<tr>
<td>Volume (ml) of gastric juice</td>
<td>7.15±0.053</td>
<td>2.2±0.035***</td>
<td>1.28±0.029***</td>
</tr>
<tr>
<td>Acid concentration (mEq/L)</td>
<td>23.94±0.365</td>
<td>14.47±0.082***</td>
<td>12.45±0.182***</td>
</tr>
<tr>
<td>Acid output (uEq/hr)</td>
<td>42.66±0.35</td>
<td>7.625±0.054***</td>
<td>6.7±0.11***</td>
</tr>
<tr>
<td>Pepsin concentration (mg/ml)</td>
<td>0.52 ±0.007</td>
<td>0.28 ±0.003***</td>
<td>0.243±0.024***</td>
</tr>
<tr>
<td>Mucus concentration (mg/ml)</td>
<td>0.307±0.004</td>
<td>0.473±0.0025***</td>
<td>0.96±0.0057***</td>
</tr>
</tbody>
</table>

*** Significant at P < 0.001
REFERENCES


دراسة تأثير الأميبيرازول والرابينتيدين على قرح وأمراض معدة الجرذان

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المكتب العلمي - شركة إيبيكو للأدوية - القاهرة

استهدف هذا البحث مقارنة تأثير كل من دواء الرانيتيند والاميبيرازول على قرح وأمراض معدة ذكور فئران البيضاء المستهدفة عن طريق اليوتير العصبي وذلك بالتنبيت على الظهر لمدة 72 ساعة وكذلك تأثير اليوتير العصبي والوقاية لهذه الأمراض.

تم إعطاء الفئران بجرعات مختلفة عن طريق الفم والحقن قبل تثبيت الفئران مباشرة وبعد مرور ساعة تم ربط الجزء البولوي للمعدة.

ثم قُتل الفئران بعد 4 ساعات وتم قياس العصارة المعدية في نبوبها مدرجة لقياس حجمها وتحليلها كيمياً اتدعوية تأثير الأدوية على تركيز الأمور تم حساب تركيز أمور في اليوتير العصبي وتركز المخاط المعدة وقد أوضح الاستنتاجات بعد تحليلها احتمالاً ماً:

- وجد أن الأميبيرازول بجرعة 0.05 ملجم/كجم من وزن الجسم عن طريق الفم والرابينتيدين بجرعة 10 ملجم/كجم من وزن الجسم عضل لها أعلى تأثير على انقص معدل النقرح، نقاط النقرح وانخفاض معدل النقرح وكذلك زيادة المعايير الوقائي بنسبة 94.2% و95% على التوالي.

- وجد أن الأميبيرازول 0.05 ملجم/كجم من وزن الجسم عن طريق الفم له تأثير أعلى من من الرانيتيند (15 ملجم/كجم من وزن الجسم عن طريق الفم) على انقص حجم العصارة المعدية وكمد الأمور حمض اليبودوكولوك.

وجد أن الأميبيرازول (بطرقية تعبيرية على الوجه) كان له تأثير أعلى من الرانيتيند في انقص أمور اليوتير العصبي في العصارة المعدية وأن الأور بالأول يزيد من تركيز المخاط بينما يقلل الثاني من هذا التركيز.

وستخلص من ذلك أن الأميبيرازول (0.05 ملجم/كجم من وزن الجسم بالفم) والرابينتيدين (10 ملجم/كجم عن طريق العضل) لهما أعلى معامل وقاية ضد الفرخة المعدية المحدثة باليوتير العصبي.

وتم تقديم هذا البحث المتعلقة بالأميبيرازول والرابينتيدين من أجل التحسن في الدراسات الجراحية في اليوتير العصبي ومنع ارتفاع الحمض ونزوله في الرئتين مما يؤدي إلى الأذى الرئوي.