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EFFECTS OF A PROTON PUMP INHIBITOR OMEPRAZOLE AND THE H_2 - 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 H_2-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 omperazole and ranitidine in doses of 5.4 mg/kg b.wt (orally) and 10 mg/kg b.wt (i.m.) respectivily . 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 H_2 -receptor antagonist. It is known to innhib gastric secretion elicited by muscarinic agonists and histamine or other H_2-agonists in a dose dependent manner and it can protect experimental animals from induced gastric ulceration [1].

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 arround 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 . Approximatelly 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]. Omperazole is excreted in urine after its metabolism in the liver . Omperazole is highly effective in healing ulcer, rapid symptoms relief and far superior to the H_2-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 - Ethene diamine, N-2 [2- [[[5 [dimethylamino) - Methyl] -2- Furanyl] Methyl] thio] ethyl] -N- methyl -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 omperazole 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 [carboxymethylcellalose 1% suspension, CMC] 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 omeperazole 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 (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 [7]. The number and severity of discrete areas of damage in the glandular mucosa 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 .

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The preventive effect of any of the antiulcer agents used was calculated according to reported method [10] .

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

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 [11]. 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 Kiled 4 hours after pylorus ligation [12]. The gastric juice was taken, measured and centrifuged, the supernatent fluid was analysed for titritable acidity [13], proteolytic acidity of pepsin [14] and mucous concentration [15] standard calibration curve (Fig. 1) showing the mean optical density of various pepsin concentrations at 280 mμ using casein substrate aquous solution 1% of trichloroacetic acid 6% (Table 1 and Fi. 1).

Different concentrations of equal amounts of d-galactose and d-mannose strarting from 0.1-0.7 mg /1 ml were prepared [16] and standard calibration curve showing the mean optical density of varous hexoses concentration at 500 mu (Table 2 and Fig. 2) .

RESULTS AND DISCUSSION

Rats are susceptible to stress induced gastric ulceration which is histologically similar to human stress ulcer [17]. 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 [18].

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 [9].

The propable mechanism of stress ulcer may be the increase of gastric motility by stress resulting in mechanical rubbing of gastric mucosa, [19]. Furthermore , the increase of acidity may be associated with vagal over-activity [20] and blocking of the ischaemic effect protects aganist the gastric stress ulceration . The release of thyrotropin releasing hormone from the stomach wall (Which is mediated by both muscarinergic and histaminergic H_2 system) is important in the pathogenesis of stress ulcer [21] .

Effect of ranitidine and omeprazole on stress induced gastric ulcration :

Ranitidine decrease the incidence of gastric ulceration and significantly decrease the mean ulcer score and ulcer index in male albino rats (15,30 mg/kg b.wt orally and 5-10 mg/kg b.wt. i.m.) .

The drug significantly increase the preventive index aganist stress ulceration (Table, 3) . It was found also that omeprazol (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 aganist gastric ulceration. These results are in accordance with that reported before [21].

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 proteotytic activity of pepsin (Table 4).These results are in accordance with that reported before [22]. 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 berfore [23], 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 [24].

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 abtaind before [25].

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 anaesthia to avoid incidence of stress ulcer .

Table (1) : Optical density of various pepsin concentrations at a wave length 750 nm.

Pepsin concentration (mg/ml)	Mean optical density* ± S.D.
0.1	0.13 ± 0.003
0.2	0.18 ± 0.001
0.3	0.22 ± 0.001
0.4	0.27 ± 0.001
0.5	0.31 ± 0.01
0.6	0.35 ± 0.02
0.7	0.39 ± 0.015

* Mean of 3 experiments for each concentration

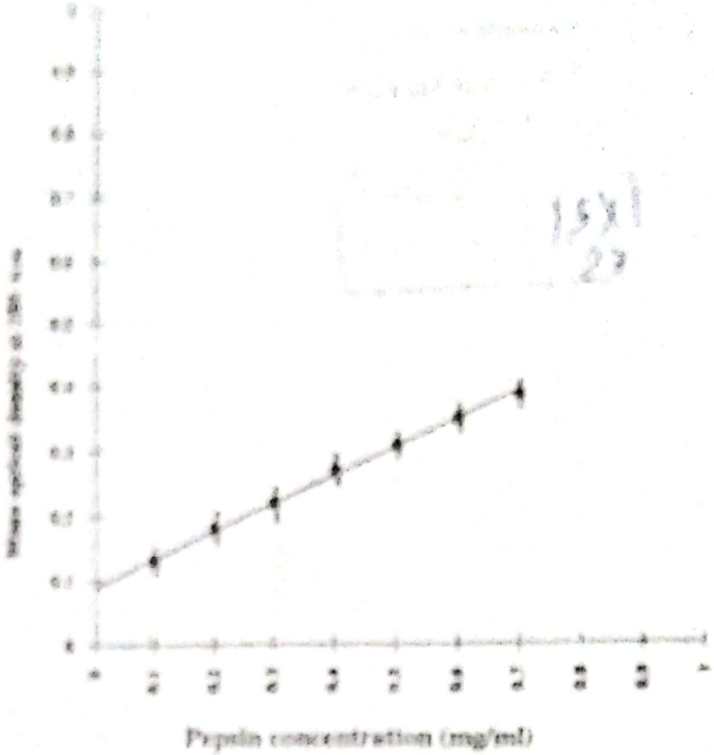

Fig. (1) : Standard calibration curve showing the mean optical density of various pepsin concentrations at 750nm.
* Vertical lines represent the standard deviation of the mean.

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

Hexose concentration (mg/ml)	Mean optical density ± S.D.
0.1	0.01 ± 0.003
0.2	0.1 ± 0.028
0.4	0.25 ± 0.035
0.6	0.4 ± 0.028
0.7	0.48 ± 0.025

* Mean of 3 experiments for each concentration

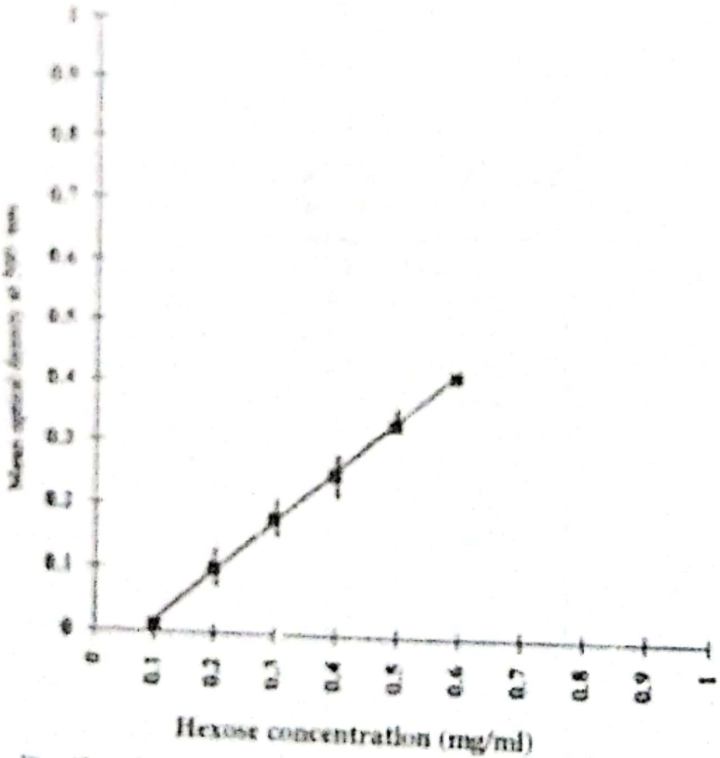

Fig. (2) : Standard calibration curve showing the mean optical density of various Hexose concentrations at 500nm.
* Vertical lines represent the standard deviation of the mean.

Table (3) : Effect of ranitidine and omeprazole on the incidence of gastric ulceration, mean ulcer score, ulcer index and preventive index in immobilized rats .

drug mg/kg / Parameters	Control	Omeprazole			Ranitidine			
		1.8 mg/kg orally	3.6 mg/kg orally	5.4 mg/kg orally	5 mg/kg orally	10 mg/kg orally	15 mg/kg orally	30 mg/kg orally
Incidence of gastric ulceration	100%	60%	60%	40%	80%	40%	90%	80%
Mean ulcer score	3.2±0.07	1.0±0.035***	0.80±0.13***	0.6±0.07***	1 ±0.07***	0.4±0.025***	1.4±0.07***	1 ±0.13***
Ulcer index	320	60	48	24	80	16	126	80
Preventive index	-	81.25%	85%	92%	75%	95%	60.5%	75%

*** 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 .

drug mg/kg / Parameters	Control	Omeprazole			Ranitidine	
		1.8 mg/kg orally	3.6 mg/kg orally	5.4 mg/kg orally	5.4 mg/kg orally	5.4 mg/kg orally
Volume (ml) of gastric juice	7.15±0.053	2.2±0.035***	1.28±0.029***	1.1 ±0.0035***	3.175± 0.04***	3.875±0.041*
Acid concentration (mEq/L)	23.94±0.365	14.47±0.082***	12.45±0.182***	7.15±0.182***	16.21 ± 0.3***	18.81± 0.15**
Acid output (uEq/hr)	42.66±0.35	7.625±0.054***	6.7 ± 0.11***	3.775±0.054***	12.475±.065***	17.96±0.115
Pepsin concentration (mg/ml)	0.52 ±0.007	0.28 ±0.003***	0.243±0.024***	.146 ± .0013***	.205 ±0.002***	0.343±0.014*
Mucus concentration (mg/ml)	0.307±0.004	.473±0.0025***	0.96±0.0057***	1.357± 0.004***	0.19±0.0035***	0.227±0.004*

*** Significant at P < 0.001

REFERENCES

23- **Blandizzi C., Gherardi G., Natale G., Marveggio C., and Deltacca M. :** Protective action of omeprazole against gastric Mucosal injury induced by hemorrhagic shock in rats., **Digestive Diseases & Sciences,** 39 (10) : 2109 - 2117 (1994).

1- **Brogden R.N., Carmine A.A., Hell R.C., Speight T. M. and Avery G. S. :** Ranitidine : a reviw of its Pharmacology and therapeutic use in peptic ulcer disease and other allied diseases . **Drugs,** 24, 267-303 (1982).

2- **Lauritsen K.:** Clinical pharmacokinetics of drugs used in treatment of gastrointestinal disease . **Clin. Pharmacokinet.** : PP. 11-13 (Part I) and PP. 64-125 (Part II) (1990).

3- **Frylund J., Gedda k., and Wallmark B. :** specific labelling of gastric H^+-K^+ ATpase by omeprazole. Biochemical **Pharmacology.** 37 (13), 2543- 9 (1988).

4- **Reynolds J. E. F. (1993) :** Martindale (The extra Pharmacopoeia) 30th edition, the Pharmaceutical Press. London .

5- **Lundell L, Backman L., Ekstrom P., Enander L. K., Falkmer S., Fausa O., Grimelius L., Havu N., Lind T. and Lonroth H. :** Prevention of relapse of reflux esophagitis after endoscopic healing the efficacy and safety of omeprazole compared with ranitidine. **Scand. J. Gastroenterology,** 26, (3), 248-56 (1991).

6- **Shay H., Komarov S. A., Fels S.S., Meranze D., Greunstein M. and Sliplet H. :** A simple method for uniform production of gastric ulceration in the rat. **Gastroenterology,** 5, 43- 61(1945).

7- **Brodie D.A., Marshall R.W. and Moreno O.M.:** Effect of restraint on gastric acidity in the Rat. **Am. J. Physiol.,** 202, 812 - 816 (1962).

8- **Wilhelmi G., and Menasse -Gdynia R. (1972) :** Pharmacology, 8 : 321-328 . Quoted from Sadik, S.A.E. (1984) : Studies on the effect of some antidepressant drugs on gastric secretion and ulceration in rats. M.sc. . thesis in Pharmacology, Al Azhar University, (Egypt).

9- **Radwan A. G., and West G.B. : Br. J. Pharmac, Chemother.** 41 : PP. 167-169 . Quoted from Sadik, S.A.E. (1984) . Studies on the effect of some antidepressant drugs on gastric secretion and ulceration in Rats. M. Sc thesis in pharmacology Al-Azhar University (1971).

10- **Radwan A. G. and Ghaleb, H. A. :** The effect of doxepin and benzuin a mide is comparison with chlorpromazine on gastric acid secretion and ulceration in the rat A. P- **Azhar Med. J.** (Egypt), 3 (1): 85-90 (1974).

11- **Robert A., Nezamis J. E., Lancaster C. and Hanchar A. J.:** Cytoprotection by prostaglandins in rats. **Gastroenterology,** 77, 433-443 (1979).

12- **Levine F. F. : Scand J. Clin. Lab. invest.,** B, 303 (1965).

13- **Grossman M. I. (1963) :** In : "Physiology for Physician ". Am. physiological association, washington (7), P.i. Brodie, D. A. (1966) . The mechanism of gastric hyperacidity produced by pyloric ligation in the Rat. **Am. J. Dig. Diseases.** 11, PP. 231-41 .

14- **Jrgensen M. B. :** A modified method for the determination of pepsinogen in urine, **Scand J. Clin. Lab -invest.,** 6, 303 (1954).

15- **Sheltar M. R., Foster J. V. and Everett M. R. :** Detrmination of serum polysaccarides by the Tryptophane reaction proc. of the society for exp. Biology and Medicine . Vol. 67. No. 2. 125 - 130 (1948).

16- **Mocazor E. and Edward W. : Journal of Chromatography.** 181, 108-114 (1980).

17- **Brodie D.A. and Hanson H.M. : Gastroenterology,** 38, 353 (1960).

18- **Ghash M.N. (1971):** Rat as an experimental animal. Fundamentals of Experimntal Pharmacology Scientific Book Agency. p. 4 .

19- **Yono S. Akahane M. and Harada M. :** The role of gastric Motility in the development of stress induced gastric Lesions of rats. **Jap- J. Pharmacol.,** 28, 607 - 615 (1978).

20- **Yano S., Nakajona S. and Harada M. :** Autodigestive susceptibility of rat gastric Mucosa. in the early stage of stress ulceration. **Jap. J. Pharmacol.,** 27, 118 - 120 (1977).

21- **Uchida A., Mitsuma I., Morise k., Kaneko H., Nagai H., Furusawa A., Nakada K. and Maeda Y. :** The role of thyrotropin-releasign hormone (TRH) in the pathogenesis of water immersion stress in rats - inhibition of TRH release from the stomach. by atropine, ranitidine, or omeprazole. **Gastroenterologia** , Japonica . 28 (1), 1-19 (1993).

22- **Miyake H., Fukuda K., Masuda Y. and Okabe S. :** Effects of a proton pump inhibitor omeprazole on healing of acetic acid -induced gastric ulcers in rats . **Gastroenterology,** 88, 1504 (1985).

24- **Douglas W.W. (1985):** Histamine and 5-hydroxytryptamine (serotonin) and their antagonists in : Goodman and Gilman : the pharmacological Basis of therapeutices . 6th ed. Gilman, A.G.; Goodman, L.S.; Rall, T.W. and ferid murad (eds.) New Youk. Macmillan publishing company. P. 605 -634 .

25- **Matsumato A., Asada S., Okumura Y., Takiuchi H., Hirata I., and Ohashilba S. :** Effect of anti -acid secretory agents on various types of gastric Mucus -in. **Journal of Clinical Gastroenterology,** 14, suppl. 1, PP. 594- 7 (1992).

دراسة تأثير الأوميبرازول والرانيتيدين على قرح وافرازات معدة الجرذان

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استهدف هذا البحث مقارنة تأثير كل من دواء الرانيتيدين والأوميبرازول على قرح وافرازات معده ذكور لفئران البيضاء المستحدثه عن طريق التوتر العصبى وذلك بالتثبيت على الظهر لمدة ٢٤ ساعة وكذلك تحديد التقرحات المعدية وحساب معدلها ونقاط التقرح ومعامل التقرح والوقاية لهذه الأدوية .

تم اعطاء العقارين بجرعات مختلفة عن طريق الفم والحقن قبل تثبيت الفئران مباشرة وبعد مرور ساعة تم ربط الجزء البوابى للمعدة . ثم قتل الحيوان بعد ٤ ساعات وجمعت العصارة المعدية فى انبوبة مدرجه لقياس حجمها وتحليلها كيميائيا لدراسة تأثر الادوية على تركيز وأفراز حامض الهيدروكلوريك وكذلك تركيز انزيم الببيسين وتركيز المخاط المعدى وقد أوضحت الدراسة بعد تحليلها احصائيا مايلى :-

- وجد أن الأوميبرازول بجرعة ٥ر٤ ملجم/كجم من وزن الجسم عن طريق الفم والرانيتيدين بجرعة ١٠ ملجم / كجم من وزن الجسم عضل لها أعلى تأثير على انقاص معدل التقرح ، نقاط التقرح ومعامل التقرح وكذلك زيادة المعامل الوقائى بنسبة ٥ر٩٢٪ و ٩٥٪ على التوالي .
- وجد أن الأدميبرازول ٥ر٤ ملجم/كجم من وزن الجسم عن طريق الفم له تأثير أعلى من الرانيتيدين (١٥ ملجم/كجم من وزن الجسم عن طريق الفم) على انقاص حجم العصاره المعديه ومعدل أفراز حمض الهيدروكلوريك .

وجد أن الاوميبرازول (بطريقة تعتمد على الجرعة) كان له تأثير اعلى من الرانيتيدين فى انقاص انزيم الببسين فى العصاره المعدية وأن الاول يزيد من تركيز المخاط بينما يقلل الثانى من هذا التركيز .

ونستخلص من ذلك أن الاوميبرازول (٥ر٤ ملجم/كجم من وزن الجسم بالفم) والرانيتيدين (١٠ ملجم/كجم عن طريق العضل) لهما أعلى معامل وقائى ضد القرحه المعدية المحدثه بالتوتر العصبى .

وننصح باعطاء المرضى الاوميبرازول والرانيتيدين قبل أجراء العمليات الجراحية بساعة واحده لمنع حدوث التقرحات الناتجه عن التوتر العصبى ومنع ارتجاع الحمض ونزوله فى الرئتين مما يؤدى الى الألتهاب الرئوى .