Rabeprazole versus Omeprazole in the protection of the gastric mucosa against NSAID induced gastric mucosal injury in rats

*Mohammed J Manna
**Majid R AL- Zaidee

ABSTRACT

Objective:

To investigates the nitric oxide donating properties of furoxan moiety and validates its role in the gastroprotective effect of rabeprazole against indomethacin induced gastric mucosal damage.

Methods:

: The study was performed between April and July 2010 in the Department of Pharmacology / College of Medicine /Baghdad University .The study was conducted on 72 adult male albino rats, divided into 6 groups, the first served as a control received the vehicle , the second received indomethacin orally of 60mg/kg. The third and fourth groups were pretreated orally 30 minute prior indomethacin with either rabeprazole or omeprazole. In order to study the possible role of nitric oxide (NO) in their gastroprotective effect ; intraperitoneal N^G-L-Arginine Methyl Ester (L-NAME) a nitric oxide synthase inhibitor was given 30 minutes prior to rabeprazole and omeprazole administration followed by indomethacin and this served as fifth and sixth group respectively .

The rats were then sacrificed after 4 hours and their stomachs were isolated and submitted to macroscopical assessment and for the measurement of the gastric prostaglandin E2 (PGE2), and myeloperoxidase (MPO) .

Results:

Rabeprazole and omeprazole produced significant gastroprotective effects. Their protective effects were associated with marked decrease in MPO activity. However, the protective effect of furoxan containing rabeprazole was significantly better than that of in omeprazole .On the other hand, L-NAME pretreatment decreased the effects of rabeprazole while L-NAME pretreatment don't decrease the protective effects of omeprazole.

Conclusions:

The prophylactic use of rabeprazole and omeprazole in this study prevented indomethacin induced gastropathy. However, protective effect of rabeprazole was significantly better than that of in omeprazole which indicates the important role of NO in the protective effect of rabeprazole.

* MSc. Pharm.Al-Sadir general hospital
**FICMS,Al-Sadir general hospital

Introduction:

It was reported that incorporated of nitric oxide-releasing properties into a NSAIDs may minimize or protect against NSAIDs induced gastropathy[1][2] .Nitric oxide (NO) is a crucial mediator of gastrointestinal (GI) mucosal defense[3] , exerting many of the same actions as prostaglandins (PGs) in the GI tract [4] in addition it is capable of inhibiting neutrophil adherence and activation and mast cell degranulation [5] , and has a number of effects in the GI tract that could counteract the loss of protective gastric prostanoids by NSAIDs mucosa [6] , increases blood flow to the gastric mucosa and maintain its integrity and defense [7] , promoting repair and removal of toxins [8] , decreases interaction of neutrophils with the gastric microcirculation [1] , and may promote the healing of gastric ulcers[9]. Rabeprazole , a newer proton pump inhibitor (PPI) provides reliable control of gastric acid secretion with more potent antisecretory activity than that of other PPIs such as omeprazole , and lansoprazole [10] .In addition to , rabeprazole contains a furoxan moiety [11 , 12] that has the ability to release NO and reduces histamine secretion [1] .In addition , early reports showing that furoxan moiety can mimic some of the physiological actions of NO [13] . In this study the gastroprotective role of two different PPIs rabeprazole a furoxan containing drug and omeprazole against indomethacin induced gastropathy was investigated and their effects on PGE2 production , NO release and MPO activity were evaluated .

Methods:

This study was conducted on 72 adult male albino-Wister rats weighing (200-250 g) , divided into 6 groups, each group carried out with 12 rats per treatment. The study was initiated after seeking approval from the ethical and scientific committee in the Department of Pharmacology / College of Medicine /Baghdad University on April 2010 . Rats were starved for at least 24 hours before indomethacin administration. During starvation , rats were kept in cages provided with a wide wire - mesh floor to avoid coprophagy but allowed free access to tap water . On the day of the experiment, water was held two hours before the procedure. Indomethacin 60 mg/kg was used for the induction of gastric damage at a concentration of 15mg/ml. Indomethacin was dissolved in a vehicle of 0.9% NaCl containing tween 80 and 1% carboxy methyl cellulose (CMC). Rabeprazole and omeprazole were dissolved each in the vehicle and their concentrations were adjusted to 10mg/ml. L-NAME was dissolved in phosphate buffer saline (PH 7.2) at a concentration of 32.5 mg/ml for intraperitoneal (I.P) administration according to the method of Griffith and Kilbourn, 1996 [14].All drugs were freshly prepared immediately before use. The animals were divided into six groups the first group served as a control received the vehicle , the second group received indomethacin orally of 60mg/kg .The third and fourth groups were pretreated orally 30 minutes prior indomethacin with 40mg/kg of either rabeprazole or omeprazole .In order to study the role of NO in the protective effect, intraperitoneal L-NAME 20mg/kg was administered 30 minutes before rabeprazole and omeprazole and served as the fifth and sixth groups respectively . At the end of each experiment (4 hours following indomethacin administration) the rats were sacrificed and their stomachs were isolated .Stomachs were opened along the greater curvature and the lengths of ulcerative lesions were measured with a digital caliper and the stomach then quickly divided into two parts and each part was kept in suitable and special buffer and stored at -20°C for biological assay.

Assessment of gastric mucosal damage:

Gastric damage score was calculated by the summation of the lengths of all linear erosions according to Santucci, et *al.* (1994) [15]

Biological assays:

Gastric mucosal samples were collected each in specific buffer and stored in freeze

until evaluation of biological parameters:

A :prostaglandin E2 assay: The samples used for assay of PGE2 were kept in sodium phosphate buffer (10 mmol/l ; pH 7.4).At the time of the procedure, tissue was minced with scissors, placed in a shaking water bath at (37°C) for 20 min, then samples were centrifuged at (9000 x g) for 1 min the concentration of PGE2 in the supernatant was determined by enzyme linked immunosorbent system (ELISA) using commercially available kit according to Wallace, *et a l.*(2000)[16]

B: Gastric MPO activity assay : The samples used to assay gastric MPO were kept in phosphate buffer saline (50 mmol/l ; pH 6) .One hundred milligram of gastric tissue was homogenized in 2 ml of PBS (50 mm) containing 0.5% hexadecyl trimethyl ammonium bromide (HTAB) (pH 6). Each sample was homogenized on ice bath for 2 minutes using a polytron homogenizer and then centrifuged at 2000 x g for 5 min. at 4°C. MPO activity of supernatant was determined by adding 0.1 ml of the supernatant to 2.9 ml of 50 mm phosphate buffer containing 0.167 mg/ml of O-diansidine HCl and 50 µ l of 1% H_2O_2, the change in absorbance at 460 nm over a 3 minutes period was measured spectrophotometrically. One unit of MPO activity was defined as that which would convert 1 Mmol of H_2O_2 to water in 1 min. at 22°C. The results were reported as the MPO unit /mg of tissue according to Bradley, *et al. (1982)*[17]

Statistical analysis:

The data were managed and analyzed using SPSS version 11 (SPSS, Inc, Chicago, IL, USA). The means, standard errors, standard deviations, 95% confidence intervals, and data range were used as descriptive statistics. One way ANOVA test was used for comparison between groups of control, indomethacin, rabeprazole with and without L_NAME pretreatment, and omeprazole with and without L_NAME pretreatment.

The between-group differences in the means of gastric damage score, myeloperoxidase enzyme and prostaglandin E2 levels were analyzed using post hoc Scheffe test. All statistical tests were two-tailed with a p value of < 0.05 deemed statistically significant.

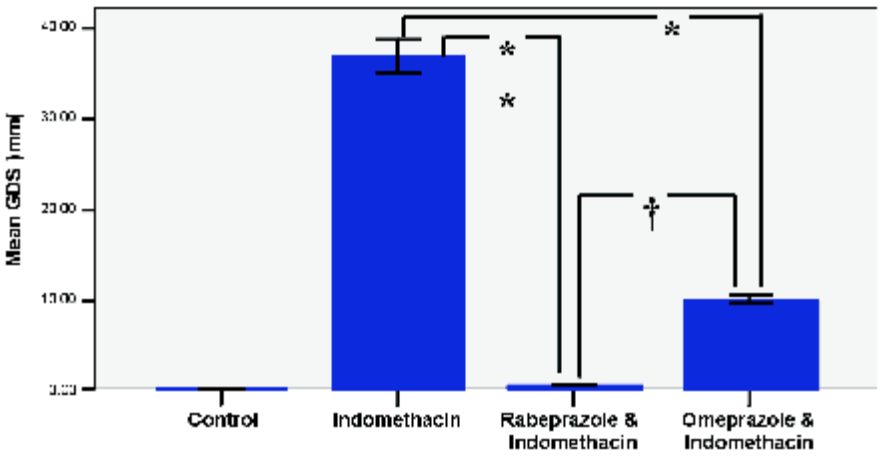
Results:

Indomethacin treated group: Intragastric instillation of 60 mg/Kg indomethacin on empty stomach, caused extensive multiple hemorrhagic lesions affecting mostly the glandular portion of the stomach in all animals which were observed 4 hrs after indomethacin administration compared with a normal gastric mucosa in control group. Indomethacin caused a significant ($p< 0.05$) mucosal injury represented by a gastric damage score (GDS) of mean (36.89$\pm$ 0.89mm) when compared with control group as shown in Figure (1). In addition indomethacin caused significant suppression ($p<0.05$) of gastric PGE2 mean (57.92 $\pm$ 1.56ng/g) versus (222.08 $\pm$ 2.92ng/g) in the control group as shown in figure (2). Also there was significant increased ($p<0.05$) in gastric MPO activity mean (35.08$\pm$ 0.83u/mg) versus (4.48$\pm$0.13/mg) in the control group as shown in figure (3). Rabeprazole pretreated group: rabeprazole pretreatment caused significant reduction ($p< 0.05$) of GDS , mean (0.52$\pm$ 0.03mm) compared to (36.89$\pm$ 0.89mm) in the indomethacin treated group as shown in figure (1) . Gastric PGE2 level was not significantly increased mean **(** 64.17$\pm$ 2.2 ng/g**)** versus (57.92 $\pm$ 1.56ng/g) in the indomethacin treated group as shown in figure (2) .By evaluating the effect of rabeprazole on MPO activity ; there was significant decrease ($p< 0.05$) in MPO activity mean (6.27$\pm$ 0.28 u/mg) compared to (35.08$\pm$0.83u/mg) in the indomethacin treated group as shown in figure (3) . When L-NAME is given 30 minute prior to rabeprazole in order to validate any role of NO ,L-NAME pretreatment causes significant decrease in the gastroprotective effect of rabeprazole ,

GDS mean (8.30± 0.19 mm) compared to (0.52±0.03mm) in rabeprazole alone treated group as depicted in figure (4). Omeprazole pretreated group: Omeprazole pretreatment also demonstrate significant gastroprotective action against indomethacin induced gastropathy ; GDS (10± 0.26mm) compared to (36.89± 0.89mm) in the indomethacin treated group as shown in figure (1).This effect was correlated with the inability of omeprazole to up regulates gastric PGE2 level mean **(** 59.58± 1.44 ng/g **)** versus (57.92 ± 1.56ng/g) in the indomethacin treated group as shown in figure (2).

In addition omeprazole demonstrate significant change in gastric MPO activity mean (10.46± 0.34 u/mg) compared to (35.08± 0.83u/mg)) in the indomethacin treated group as shown in figure (3) .

On the other hand L-NAME pretreatment failed to decrease the cytoprotective effect of omeprazole; where it is shown that there is no significant effect on GDS as shown in figure (4) . However , by comparing the cytoprotective action between rabeprazole and omeprazole ; reveals that rabeprazole was more effective in prevent ulcerogenic action of indomethacin , where rabeprazole causes significant decrease in GDS of (0.52± 0.03mm) compared to (10± 0.26mm) in omeprazole treated group as shown in figure (1), and its suppression of MPO activity was significantly greater than that of in omeprazole as shown in figure (3) .



Fig(1) : The effect of rabeprazole versus omeprazole pretreatment on the gastric damage score induced by indomethacin. The results are expressed as the mean ± SEM

* P < 0.05 when compared with indomethacin group.

†

P < 0.05 when compared with omeprazole pretreated group.

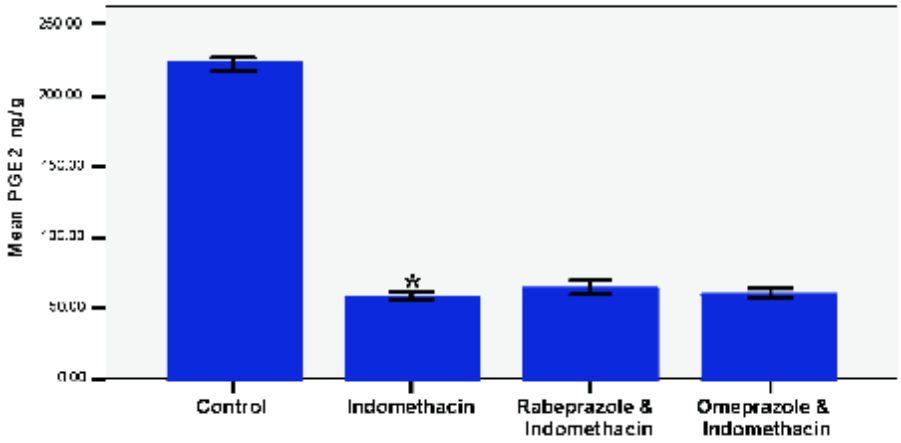


Fig.(2) : The effect of rabeprazole versus omeprazole pretreatment on the gastric PGE2 levels inhibited by indomethacin. The results are expressed as the mean ± SEM.

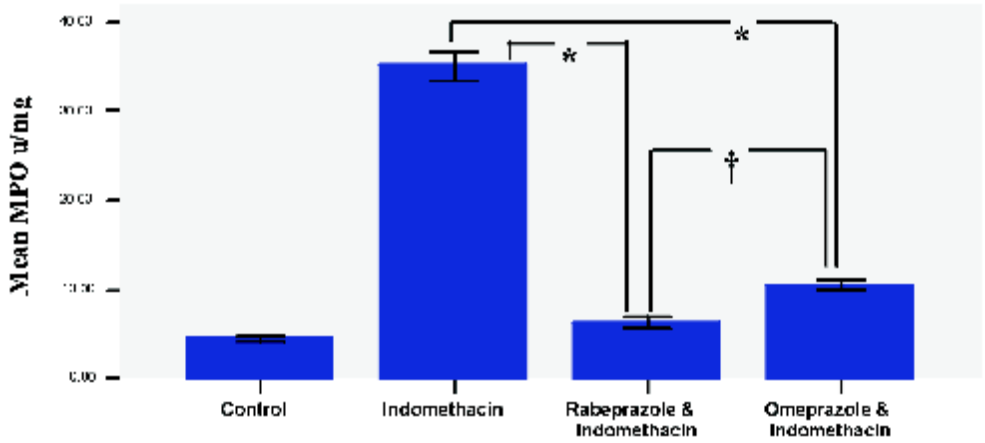
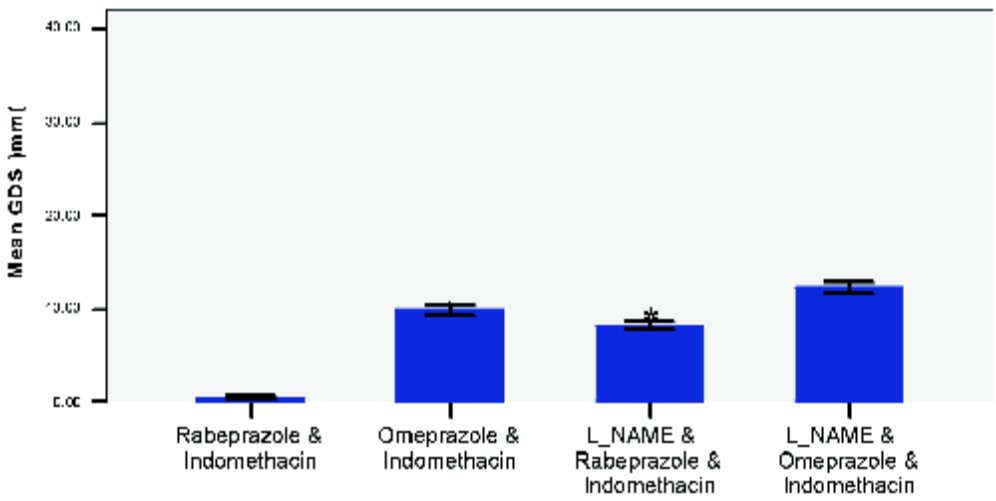


Fig.(3) : The effect of rabeprazole versus omeprazole pretreatment on the increased gastric MPO activity induced by indomethacin .The results are expressed as the mean ± SEM .

* P < 0.01 when compared with indomethacin group.

†P < 0.01 when compared with omeprazole pretreated group.



Fig(4) : The effect of L-NAME pretreatment on the cytoprotective effect of rabeprazole and omeprazole on the gastric damage score induced by indomethacin . The results are expressed as the mean ±

* P < 0.05 when compared with rabeprazole pretreated group without L-NAME.

Discussion:

PPIs are now approved for the treatment and prevention of recurrence of NSAIDs-induced gastric damage in patients who continue NSAIDs use [18] . This therapeutic effect of PPIs is generally attributed to their potent inhibitory effects on acid secretion through inhibition of the gastric H,K-ATPase [19] However ,in this study the significant reduction in the gastric damage score obtained could not be explained on this bases alone . This is because in present study only one single dose of rabeprazole and omeprazole were used while the maximum suppression of acid secretion requires several doses of PPIs [18] . Moreover the time allowed of PPIs to produce its effects on acid secretion in this study was relatively short (4.5 hrs) , where more than 5 hrs are needed before any significant increment in the gastric acid PH could be detected [20]

In this study pretreatment with rabeprazole and omeprazole elicited a significant decrease in the extent of the gastric damage caused by indomethacin. Their ability to protect the gastric mucosa did not seem to be related to gastric PGs since omeprazole and rabeprazole in this study were not able to alter the gastric PGE_2 level which was significantly suppressed by indomethacin. However, this protective effect was associated with significant inhibition of MPO activity, a specific marker for oxyradical generation , neutrophil infiltration in tissues and adherence to the vascular endothelium, which are the early events of gastric damage associated with the use of NSAIDs [21] .

This reduction of MPO activity is one of the cytoprotective effects of omeprazole and rabeprazole on the gastric mucosa .Although the general mechanism of omeprazole is similar to that of rabeprazole [18] , however , cytoprotective effect of rabeprazole in this study was significantly

Better than omeprazole .The differences observed between rabeprazole and omeprazole in their extent of protection the gastric mucosa in this experiment could be partly explained on the role of furoxan moiety that has the ability to release NO , a mediator that involved in the cytoprotection effect , where this study demonstrate important role of NO in the protective mechanisms of rabeprazole , this is because the administration of L-NAME significantly decrease the protective effects of this drug. Whereas , L-NAME pretreatment did not seem to decrease the protective effects of omeprazole on the gastric damage score and therefore any role of NO in omeprazole gastroprotection can be excluded .Another possible explanation is based on the pharmacokinetic profile of these two PPIs, where the bioavailability of omeprazole appears to increase with multiple dosing , rising from 35% for a first dose to 60% after repeated dosing [23 , 24] in contrast bioavailability of rabeprazole does not appear to change appreciably with multiple dosing [19] , this can result in greater antisecretory effects of rabeprazole during the first day of therapy[19]

References:

1. Miller MR , and Megson IL . Recent developments in nitric oxide donor drugs .British Journal of Pharmacology .2007 ; 151: 305-321 .
2. Calatayud S , Sanz MJ , Canet A , *et al* . Mechanisms of gastroprotection by transdermal in the rat . Br J Pharmacol .1999 ; 127 : 1111-1118.
3. Souza M H L P , Paula Lemons H , Oliveira RB , Cunha FQ . gastric damage and granulocyte infiltration induced by indomethacin in tumor necrosis factor 1 or inducible nitric oxide synthase deficient mice . Gut . 2004 ;53: 791-796.
4. Wallace JL , Miller MJ : Nitric oxide in mucosal defense : a little goes a long way . Gastroenterology , 2000 , 119 : 512-520.
5. Carmelo Scarpignato and Richard H Hunt Proton pump inhibitors: the beginning of the end or the end of the beginning ?. current opinion in pharmacology .2008 ; 8: 677-684.
6. Brown JF , Keates AC , Hanson PJ , Whittle BJ . Nitric oxide generators and cGMP stimulate mucus secretion by rat gastric mucosal cells . Am J Physiol.1993 ; 265 : G418-G422.
7. Full Young Chang , Chih Yen Chen , Ching Liang Lu , Jiing Chyuan Luo , Rei-Hwa Lu Shou-Dong Lee. Response of blood endothelin-1 and nitric oxide activity in duodenal ulcer patients undergoing *Helicobacter Pylori* eradication. World Journal Gastroenterology .2005 ; 11(7): 1048-1051.
8. Hallas J , Dall M , Andries A , Andersen BS Aalykke C , Hansen JM , *et al*.Use of single or combined antithrombotic therapy and risk of serious upper gastrointestinal bleeding :population based case control study .Br. Med J .2006;34 :383-398.
9. Ma L ,Wallace JL .Endothelial nitric oxide synthase modulates gastric ulcer healing in rats. Am J Physiol Gastrointest. Liver Physiol.2000; 279 : G341-G346.
10. Yuji Mizokami. Efficacy and safety of rabeprazole in non-steroidal anti-inflammatory drug-induced ulcer in Japan. World J Gastroenterol . 2009; 15(40): 5097-5102.
11. Sobra G , Galli U , Cena C , Fruttero R , Gasco A , Morini G , *et al* .A new furoxan NO-donor rabeprazole derivative and related compounds . Chembiochem . 2003 ; 4 :899-903.
12. Saha JK , Wang T , Stewart R , Trocha M, Shumway M , Garvey D , Letts LG, Wolfe M M , Tam S W . Enhanced gastroprotective and anti-ulcerogenic activities in rats of a new class of proton pump inhibitor containing nitrosothiol nitric oxide donor. Gastroenterology . 2001; 120 (1) : A 144-A 145.

13. Gasco A, Fruttero R , Sobra G , Di Stilo A , and Calvino. Nitric oxide donors : focus on furoxan derivatives .Pure Appl. Chem. 2004 ; 76 (5):973-981.
14. Griffith O.W, and Kilbourn R.G. Nitric oxide synthase inhibitors , amino acids. methods .J. enzymol. 1996; 268 :375-392.
15. Santucci L, Fiorucci S. and Giansanti M. Pentoxifylline prevents indomethacin-induced acute gastric mucosal damage in rats. Role of TNF-, Gut. 1994; 35: 909-915.
16. Wallace J.L, McKnight W, Reuter B.K ,and Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both COX & COX-2. J. Gastroenterol. 2000; 119:706-714.
17. Bradley P.P, Christensen R.D ,and Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation. Blood . 1982 ;60: 618-622.
18. Hoogerwerf WA and Pasricha PY . Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease. In: Goodman and Gilman's the pharmacological basis of therapeutics. (Laurence L Brunton. John S Lazo and Keith L. Parker.) 2006 , section VI , ch:36. McGraw Hill : 969-974.
19. Spechler S J: Peptic ulcer disease and its complications. In: Sleisenger and Fordtran's Gastroenterology (Mark Feldman, Lawrence Friedman and Marvin H Sleisenger), 2002 , 7th edition, ch.40. SAUNDERS : 747-753.
20. Mohammed J Manna and Samir Y Matloub. Cytoprotective actions of omeprazole in indomethacin induced gastric mucosal injury in rats. J Of The Faculty Of Medicine Baghdad. 2010 ;52 (1): 79- 83.
21. Cuzzocrea S, Sautebin L , De Sarro G *et al* . Role of IL-16 in the pleurisy & lung injury caused by Carragreenan. J Immunol. 1999 ;163: 5094-5104.
22. Cederberg C , Anderson T , Skanberg I : Omeprazole : Pharmacokinetic and metabolism in man . Scand J Gastroenterol .1989 ;166 : 33-40 , .
23. Andersson T , Andren T , Cederberg C , et al : Pharmacokinetic and bioavailability of omeprazole after single and repeated oral administration in healthy subjects . Br . J Clin Pharmacol .1990 ; 29 :557- 563 .
24. Yasuda S , Ohnishi A , Ogawa T , et al : Pharmacokinetic properties of E3810 , a new PPI , in healthy male volunteers .Int J Clin Pharmacol Ther .1994 ; 32:466-473 .