

Cytoprotective Actions of Omeprazole in Indomethacin – Induced Gastric Mucosal Injury in Rats.

Mohammed J. Manna * BSC, PHARM.-MSc
Samir Y. N. Matloub* MBChB, MSC -DM

Summary:

Background: Gastric mucosal injury induced by NSAIDs is a major adverse effect of this group of medications, and it is no surprise that protection against such injury has become an important challenge for a better understanding of the mechanisms involved.

Materials and methods: This study was conducted on 40 adult male albino rats, divided into 4 groups, the first served as a control received the vehicle, the second received indomethacin orally of 60mg/kg. The third was pretreated 30 minutes prior indomethacin with omeprazole 40mg/kg orally. The fourth was given intraperitoneal L-NAME 20mg/kg plus omeprazole to investigate a possible protective role of nitric oxide. The rats were then sacrificed after 4 hours and their stomachs were isolated and submitted to macroscopical, and microscopical assessment and for the measurement of the gastric prostaglandin E2, myeloperoxidase, and interleukin-4.

Results: Omeprazole pretreatment resulted in a significant decrease ($p < 0.01$) in the gastric damage score. The MPO activity was significantly decreased ($p < 0.01$), while had no effects on the gastric levels of IL-4 and PGE2. L-NAME given 30 minutes before omeprazole had no influence on the protective effects of omeprazole.

Conclusions: The injurious effect of indomethacin can be reduced by omeprazole pretreatment, and that this protective effect of omeprazole may be partly attributed to its antioxidant property reflected by the decrease in the MPO activity.

Keywords: Cytoprotection, Omeprazole, NSAIDs gastropathy

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Introduction:

NSAIDs are widely used group of medications with many clinical applications in different areas of modern medicine (1) however NSAIDs-induced gastropathy is the major problem of this group of drugs (2). Although the damaging effect of these drugs is generally ascribed to their ability to inhibit gastric prostaglandins (PGs) (3) other mechanisms which are partially or totally independent of PGs inhibition may be important, including leukocyte adherence to the vascular endothelium (4), superoxide radical formation (5), reduced nitric oxide (NO) release (6), proinflammatory cytokine production (7), inhibition of oxidative phosphorylation in mitochondria and activation of apoptosis (8). In the present study we evaluated the cytoprotective effect of omeprazole on gastric mucosal damage induced by indomethacin. We also evaluated the effect of this antiulcer agent on NO production, PGs synthesis, MPO activity, and IL-4 expression.

Materials and Methods:

This study was conducted with 40 adult male albino-Wistar rats weighing (150-200 g). Rats were starved for at least 24 hours before indomethacin administration. On the day of the experiment, water was held two hours before the procedure. Indomethacin was used for induction of gastric damage in a dose 60 mg/kg at a concentration of

15mg/ml, Omeprazole was dissolved in the vehicle of (0.9% NaCl contain tween 80 and 1% CMC) and its concentrations was adjusted to 10mg/ml. N^G-L-Arginine Methyl Ester (L-NAME) which is NOS (nitric oxide synthase) inhibitor was dissolved in phosphate buffer saline (PH 7.2) at a concentration of 32.5 mg/ml according to the method of Griffith and Kilbourn (1996) (9) for intraperitoneal (I.P) administration. All drugs were freshly prepared immediately before the use. The animals were divided into four groups the first group served as a control received the vehicle, the second group received indomethacin orally of 60mg/kg. The third group was pretreated 30 minutes prior indomethacin with omeprazole 40mg/kg orally. The fourth group received in addition intraperitoneal (I.P) L-NAME 20mg/kg. The rats were sacrificed after 4 hours following indomethacin administration and their stomachs were isolated. The lengths of ulcerative lesions were measured with a digital caliper and the stomach quickly divided into three parts and each part was kept in suitable and special buffer and stored at -20°C for biological assay. In addition full thickness pieces of the gastric corpus were stored in 10% formalin for histopathological study. 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) (10).

Biological assays: Gastric mucosal samples were collected each in specific buffer and stored in freeze until evaluation of biological parameters:

* Department of pharmacology, College of Medicine University of Baghdad.

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 al.*(2000). (11).

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 µl of 1% H2O2, 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 H2O2 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) (12)

C: IL-4 expression assay: Quantitative measurement of IL-4 was conducted using a solid phase ELISA. The samples that were used to assay gastric IL-4 were kept in phosphate buffer Saline (pH 7.4). At the time of the procedure specimens of gastric mucosal scrapings were homogenized with sample buffer and centrifuged at (1000 x g) for 15 min and the resulting supernatant diluted. Samples and standards were pipetted into the microtiter wells precoated with antibody specific for rat IL-4 and after incubation for 2hrs at 37 °C the complex was then probed with 100 ML biotinylated antibody, and washed with 350 ML wash buffer .After being washed, the retained complex was reacted with 100 ML streptavidine peroxide and incubated with 90 ML tetramethyl benzidine (TMB) reagent for spectrophotometric IL-4 quantifications according to Slomiany , *et al.*(1998) (13).

Statistical analyses: Statistical analyses were done using SPSS. All data were expressed as mean + standard error of mean (SEM). One-way analysis of variance (ANOVA–test) was used for comparison between several experimental groups. A probability value P <0.01 was considered statistically significant.

Results:

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(100%

induction) .The microscopical appearance of gastric mucosa after indomethacin administration is depicted in photo (1).Indomethacin-induced gastric mucosal injury was reduced by omeprazole pretreatment. The microscopical appearance of which is shown in photo (2) .The gastric damage score was significantly (p<0.01) reduced by omeprazole mean(8.18 ±0.39 mm) when compared to (34.71± 0.96mm) in the indomethacin treated group as shown in figure (1) .On the other hand, gastric PGE2 Level was not significantly affected mean (74.1± 1.8 ng/g) when compared to (63.9± 2.1 ng/g) in the indomethacin treated group, as shown in figure (2) . Measurement of MPO activity shows significant decrease (p<0.01) mean (10.39± 0.64 u/mg) when compared to (28.4± 0.55 u/mg) the indomethacin treated group as shown in figure (3) .While gastric IL-4 was not significantly changed, mean (23.8 ± 1.04 pg/mg) when compared to (21.9± 0.84 pg/mg) the indomethacin treated group as shown in figure (4) . L-NAME given 30 minutes before omeprazole administration had no significant effect on the gastric damage score mean (8.2± 0.4 mm) when compared to (8.18± 0.39 mm) in the omeprazole treated group figure(1) .

Histopathological Finding

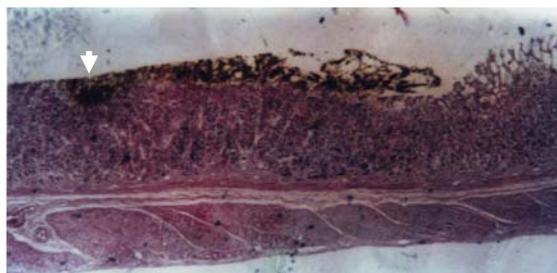


Photo (1) Microscopical appearance of the rat gastric mucosa after indomethacin (60 mg/kg) administration. Gastric mucosa shows surface degeneration change and ulcerative lesions extending beyond the muscularis mucosa with granulocyte infiltration. Paraffin section, stained (H&E), 10x.stained (H&E) 200x.

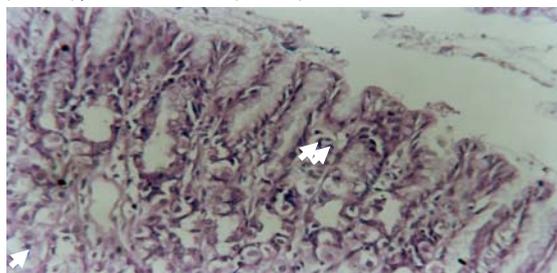


Photo (2) Microscopical appearance of the rat gastric mucosa after (40 mg/kg) omeprazole had been given 30 min. Prior indomethacin administration. Section shows normal gastric mucosa but there is hypertrophy of epithelial cells in the gastric pits mucosa (→)and moderate infiltration of inflammatory cells (↗) Paraffin section, stained (H&E) 400x.

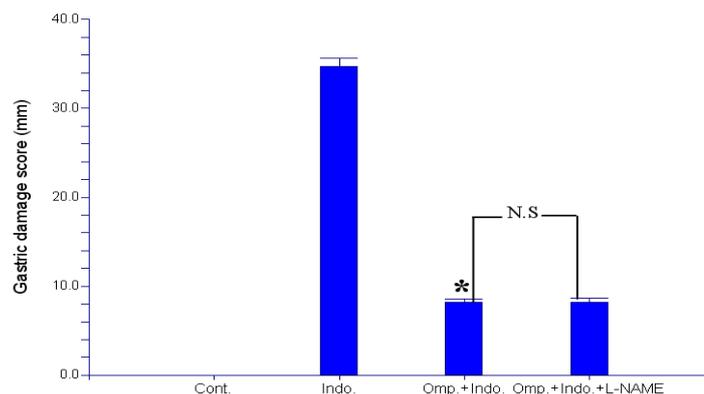


Figure (1): The effect of omeprazole with or without L-NAME administration on the reduction of gastric damage score. The results are expressed as the mean \pm SEM.

* P < 0.01 when compared with indomethacin group.

Cont: control, indo: indomethacin, Omp: Omeprazole.

NS: no significant

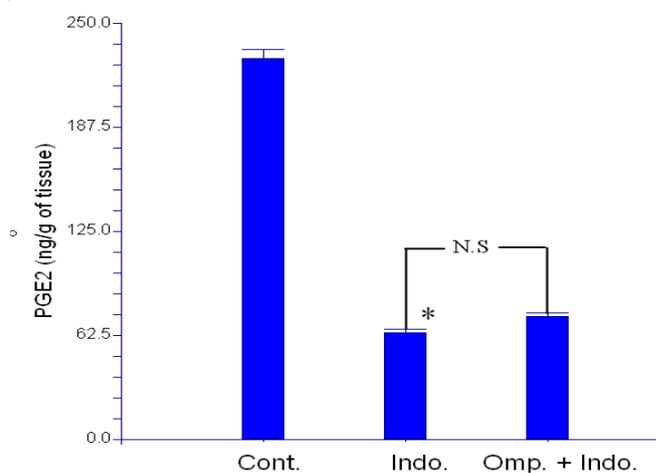


Figure (2): The Gastric PGE2 levels following omeprazole pretreatment compared with indomethacin alone showing no significant alterations. The results are expressed as the mean \pm SEM. Cont: control, indo: indomethacin, Omp: Omeprazole.

* P < 0.01 when compared with control group.

NS: no significant

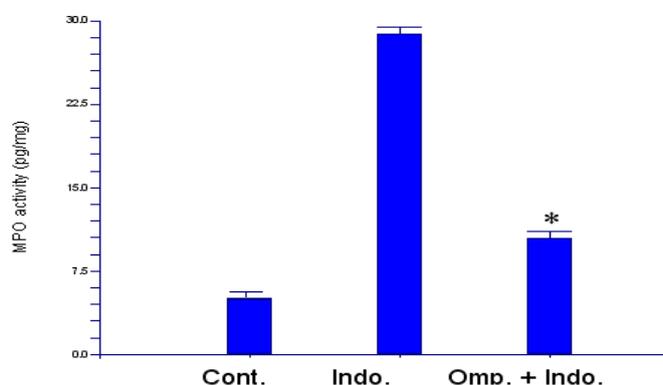


Figure (3): The effect of omeprazole on the gastric MPO activity induced by indomethacin .The results are expressed as the mean

* P < 0.01 when compared with indomethacin group.

Cont: control, Indo: Indomethacin, Omp: Omeprazole

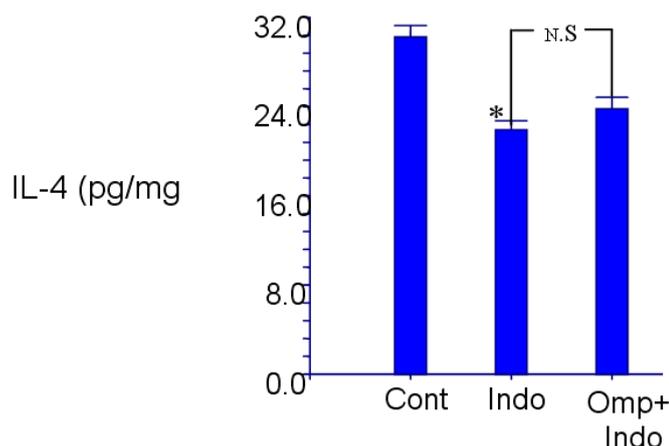


Figure (4): The effect of omeprazole on the decrease of the expression of gastric IL-4 during indomethacin induced mucosal injury, showing no significant alteration in IL-4 levels. Values expressed as mean \pm SEM. Cont: control, indo: indomethacin, Omp: omeprazole.

* P < 0.01 when compared with control group.

NS: no significant

Discussion:

Cytoprotection is defined as the ability of pharmacologic agents to prevent gastric and intestinal mucosal injury produced by a variety of ulcerogenic agents (14, 15) which is accomplished by mechanisms other than reduction of the gastric acid secretion (8). Proton pump inhibitors (PPIs) are now approved for the treatment and prevention of recurrence of NSAIDs-induced gastric damage in patients who continue NSAIDs use (16). This therapeutic effect of PPIs is generally attributed to their potent inhibitory effects on acid secretion through inhibition of gastric H/K-ATPase (16). However in this study the significant 75% 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 omeprazole was used while maximum suppression of acid secretion requires several doses (16). In addition the time allowed for omeprazole to produce its effects on acid secretion in this study was rather short (4.5 hours), where more than 5 hours are needed before any significant increment in the gastric PH could be detected according to the study of AstraMerk (1996). Moreover it has also been observed that elimination of gastric acid by an antisecretory agent does not completely prevent gastric necrosis induced by NSAIDs.(8) It has been recognized that one of the early events of gastric damage associated with the use of NSAIDs is free radical generation with neutrophil activation and its adherence to the vascular endothelium (17, 18, 19, 20). This neutrophil infiltration and oxyradical generation is reflected by the MPO activity and since this is specific to neutrophil it is often used an indicator of neutrophil infiltration in tissues' (20). In the present study omeprazole pretreatment resulted in a significant 63% reduction of this inflammatory

Marker and this reduction of MPO activity is one of the cytoprotective effects of omeprazole observed on The gastric mucosa. To investigate whether omeprazole cytoprotective effects could be through NO release ; L-NAME was used for this purpose , L-NAME in this study did not seem to abrogate the protective effects of omeprazole on the gastric damage score and therefore any role of NO in omeprazole gastroprotection can be excluded . Moreover , the ability of omeprazole to protect the gastric mucosa against NSAIDs damage did not seem also to be related to gastric PGs since omeprazole in this study was not able to alter the gastric PGE2 levels which was significantly suppressed by indomethacin .It has been shown that extensive gastric mucosal damage as that produced by indomethacin resulted in reduction of IL-4 , which is one of the anti-inflammatory cytokines that suppress the secretion of proinflammatory IL-1, IL-2, IL-6. (7) .However in this study omeprazole pretreatment failed to increase the gastric IL-4 levels which were significantly reduced by indomethacin treatment. Other cytoprotective mechanisms of omeprazole has been recently suggested and which require further study include blocking of the activation of mitochondrial and Fas receptors-induced pathways of apoptosis .(21) , mucosal cell renewal by stimulatory gene expression of proliferating cell nuclear antigen and stimulation of epidermal growth factor and basic growth factor .(21)

Conclusion:

The results of this study clearly demonstrate that the injurious effects of indomethacin can be prevented by omeprazole pretreatment, and that decreased oxyradical generation and reduced neutrophils infiltration as reflected by the reduction of MPO

activity is one of the cytoprotective effects of omeprazole.

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