A POSSIBLE ROLE FOR GASTROPROTECTIVES ON ASPIRIN-INDUCED GASTRIC ULCER IN RATS

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ABSTRACT

Background: *Gastric ulcer is a discontinuity in the gastric mucosa that occurs due to imbalance between gastric mucosal protective factors and aggressive factors.*

The Aim *of the present work was to test and compare the protective effects of an antisecretory H_2 receptor blocker; ranitidine and other recently suggested gastroprotective drugs: L-arginine; a precursor of NO, zinc sulfate; an anti-inflammatory antioxidant agent and pioglitazone; a PPAR-γ agonist, on a rat model of aspirin induced gastric ulcer.*

Methods: *Acute gastric lesion was induced in rats by a single oral dose of aspirin 300mg/ kg body weight. L-arginine 200mg / kg b. wt, zinc sulfate 80 mg/ kg b. wt, and pioglitazone 10 mg / kg b. wt. were given alone and in combination with ranitidine 50 mg / kg b. wt for 3 days before induction of gastric lesion.*

Results: *Aspirin induced a significant increase in gastric mucosal lesion score and free and total gastric hydrochloric acid with a significant decrease in gastric nitric oxide and mucin levels as compared to normal group. A significant increase in gastric malondialdhyde and decrease in reduced glutathione as compared to normal group. L-arginine, zinc sulfate, and pioglitazone produced improvement of most of the measured parameters as compared to non-treated group. Combination of L-arginine and ranitidine was superior in prophylaxis against aspirin-induced gastric ulcer when compared to the effects of each drug used individually, and the other studied combinations.*

Conclusion: *The role of HCl and NO seems more important in the pathogenesis of aspirin induced gastric ulcer, as evidenced by the better protective effects of combination of ranitidine and L-arginine in comparison to the ranitidine with either zinc sulfate or pioglitazone.*

Key words: *Pharmacology- Stomach Ulcer- Anti-Ulcer Agents- Africa, Northern, Egypt - Middle East, Egypt.*

Abbreviations:

- **NO:** Nitric Oxide
- **PPARs:** Peroxisome proliferator-Activated receptors
- **GSH**: Reduced Glutathione
- **HCl:** Hydrochloric acid
- **MDA:** Malondialdehyde
- **NSAIDs**: Non-Steroidal Anti-inflammatory drugs

INTRODUCTION

Peptic ulcer is multi-etiologic and widespread disease which is defined as a discontinuity in the gastric mucosa penetrating through the muscularis mucosa.[(1)]It usually occurs due to imbalance between the gastric mucosal protective factors, that is called the gastric mucosal barrier, and the aggressive factors, to which the mucosa is exposed. Aggressive factors promoting gastric mucosal injury include gastric hydrochloric acid HCl, mucosal hypoperfusion,[(2)] free oxygen radicals, and drugs, mainly NSAIDs.[(3)]

Reactive oxygen species especially the hydroxyl radical plays a major role in causing oxidative damage of mucosa in all types of ulcers including stress related gastric mucosal damage, non-steroidal anti-inflammatory drug-induced gastric lesions and H. pylori mediated gastroduodenal ulcers.[(4)] Gastric mucosa has several endogenous antioxidant enzymatic systems, against these injurious agents that can inhibit oxidation by scavenging ROS and so prevent their destructive action on the mucosa without requiring much energy. The antioxidant enzymatic systems include: catalase, glutathione peroxidase (GPx) and glutathione reductase (GR).[(5)]

Nitric oxide NO plays a critical role in modulating the defensive mechanisms in the gastrointestinal tract, whereby under physiological conditions, constitutive production of NO by cNOS in endothelial cells elicits the relaxation of vascular smooth muscle cells and creates a nonthrombogenic environment in the vasculature while also preventing the accumulation of adherent leukocytes in postcapillary venules.[(7)] It also participates in mechanisms maintaining the integrity of the gastric epithelium by regulating mucosal blood flow.[(8)] However, production of high amounts of NO from iNOS would produce detrimental effects in the gastrointestinal (GI) mucosa as it works as a pro-inflammatory mediator that can produce damage in the cell at different levels.[(9)] NO can inhibit SH-dependent enzymes and the subsequent peroxynitrite ($ONOO^-$) production may lead to peroxidation of

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lipids and destruction of enzymes inside the mitochondria.[10]

Aspirin, which is one of the most commonly used NSAID, is associated with gastrointestinal side effects of variable severities ranging from mild dyspepsia to severe fatal gastric bleeding. Aspirin leads to inhibition in the gastric mucosal protective factors and at the same time it increases the aggressive factors to which the mucosa of the stomach is exposed.[11]

In the present study, a rat model of aspirin induced gastric ulcer was used to test and compare the protective effects of an antisecretory H_2 receptor blocker; ranitidine and other recently suggested gastroprotective drugs: L-arginine; a precursor of NO, zinc sulfate; an anti-inflammatory antioxidant agent and pioglitazone; a PPAR-γ agonist, on the development of gastric ulcer. The effect of each one of these proposed gastroprotective drugs was tested alone and in combination with ranitidine.

METHODS

The present study was carried out on ninety male albino rats weighing 200-250grams that were housed under the same controlled environmental conditions, fed normal laboratory diet and had free access to tap water. The experimental protocol was reviewed and approved by the Ethical Committee of Alexandria Faculty of Medicine.

Animals were randomly divided into 9 groups each of 10 rats; Group I (control group): received daily oral dose of 2 %gum acacia (the vehicle) for 3 days as 5 ml/kg body weight (b. wt.).Gastric lesion was induced in all of the remaining eight groups (II-VIII) by oral administration of a single dose of aspirin (300mg/ kg b.wt.).[12]Three days before induction of gastric lesion; group II (untreated aspirin induced gastric lesion group): received the vehicle. Group III: received oral dose of ranitidine 50mg/kg b. wt /day.[13] Group IV: received oral dose of L-arginine 200mg/kg b. wt/day.[14]Group V: received oral dose of zinc sulfate 80mg/kg b. wt/day.[15] Group VI: received oral dose of pioglitazone 10mg/kg b. wt/day.[16] Group VII: received oral dose of ranitidine and L-arginine. Group VIII: received oral dose of ranitidine and zinc sulfate. Group IX: received oral dose of ranitidine and pioglitazone using the same doses as in monotherapy.

By the end of experimentation period; animals were sacrificed and the gastric juice of each stomach was collected and subjected to determinations of; **free and total gastric acidity**[17]**and mucin content**.[18]**Lesion score** of each rat stomach was calculated[19] and the glandular mucosa of the rest of gastric tissue was scrapped and subjected to the estimation of the levels of; **reduced glutathione (GSH),**[20]**malondialdehyde (MDA)**[21] **and nitric oxide (NO).**[22]

Statistical analysis:

Data were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was used to investigate normality within groups and a nonparametric Kruskal-Wallis test was used for the gastric lesion score that were found to be not normally distributed and significance among groups was determined by using post-test Conover-Inman test. Normal distribution was confirmed in all groups of other parameters and a parametric test, the Analysis of Variance, ANOVA was utilized and significance among groups was determined by using LSD, the least significant difference test. A level of $P < 0.05$ was defined as being statistically significant. Data analysis was performed using SPSS/version10 software package.

RESULTS

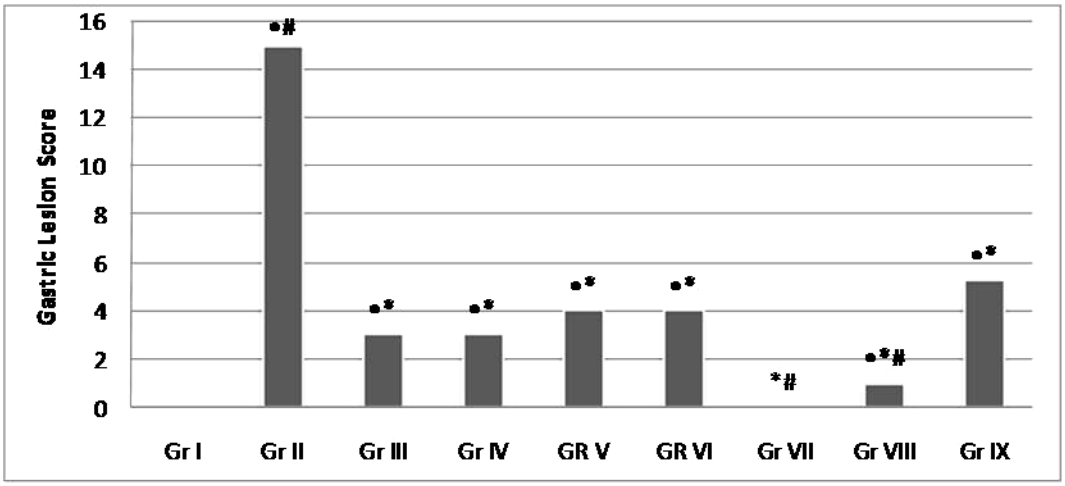
In the present study, a single oral dose of aspirin (300 mg/ kg b. wt.) produced significant increase in the gastric score lesion when assessed six hours after administration. Three-day prophylactic oral administration of ranitidine (group III), L-arginine (group IV), zinc sulfate (group V), and pioglitazone (group VI), produced a significant decrease in the gastric lesion score as compared to group II but still there were statistically significant differences between all treated groups and the normal control group. Meanwhile administration of ranitidine plus L-arginine (group VII) and ranitidine plus zinc sulfate (group VIII) produced a significant decrease in the gastric lesion score as compared to group III. However, administration of ranitidine plus pioglitazone, (group IX) produced statistically insignificant difference as compared to group III. There were statistically insignificant difference between group VII and the normal control group ($P < 0.0001$). (Figure 1)

A significant increase in both levels of free and total gastric HCL with a significant decrease in gastric mucin level as compared to normal group was also observed in aspirin untreated group. And as compared to normal control group, a significant decrease in gastric NO and gastric GSH levels with a significant increase in gastric MDA level were also shown in the aspirin untreated group. When the protective effects of the studied drugs were assessed on different studied parameters, it was apparent that ranitidine, L-arginine, zinc sulfate and pioglitazone produced improvement of most of the previously mentioned parameters when evaluated as compared to the non treated lesion group (Table I).

Except for the free gastric HCl level, combination of ranitidine with either L-arginine or zinc sulfate produced improvement of all of the previously mentioned parameters, as compared to the ranitidine-treated group. Combination of ranitidine and pioglitazone produced significant reduction in gastric mucosal lesion score and total gastric HCl

level that was accompanied by significant decrease in the level of MDA and increase in the level of GSH. No improvement of the mucin level or the NO in the gastric tissue was detected in this group as compared to ranitidine-treated rats (Table II).

Fig 1: Effect of three-day prophylactic oral administration of ranitidine (50mg/kg), alone and in combination with each L-arginine (200mg/kg), zinc sulfate (80mg/kg) and pioglitazone (10mg/kg), on gastric lesion score in aspirin induced gastric ulcer in rats (Mean ± SD).



• Significant difference in comparison to group I.* Significant difference in comparison to group II.
Significant difference in comparison to group III.

Table I: Effect of three-day prophylactic oral administration of ranitidine (50mg/kg), alone and in combination with each L-arginine (200mg/kg), zinc sulfate (80mg/kg) and pioglitazone (10mg/kg), on free and total gastric HCl level (mEq/L), and gastric mucin level (mg%)in aspirin induced gastric ulcer in rats(Mean ± SD).

Groups	Free HCL	Total HCL	mucin
I	22.5± 1.84	67.5 ± 1.35	20.46 ± 0.66
II	31.5± 3.63•	77.8± 2.62•	10.08± 0.45•
III	2.5± 1.72*	24.10± 1.45*	11.9 ± 0.83•*
IV	22.9± 1.29*	56.50± 1.08*	17.82 ± 0.9•*
V	26.3± 1.89*	63.00 ± 1.7*	17.25 ± 0.67•*
VI	27± 2.94*	71.6 ± 1.075*	12.43± 0.57•*
VII	1.1± 0.74•*	18.20 ± 1.3•*#	19.01± 0.89•*#
VIII	1.9 ± 0.10•*	20.10± 0.99•*#	19.06± 0.79•*#
IX	2.0 ± 0.94•*	21.50± 1.51•*#	12.73 ± 0.73•*
p	<0.001	<0.0001	<0.0001

• Significant difference in comparison to group I.* Significant difference in comparison to group II.
Significant difference in comparison to group III.

Table II: Effect of three-day prophylactic oral administration of ranitidine alone and in combination with each of L-arginine, zinc sulfate and pioglitazone on mucosal NO (μmol / gm tissue), MDA (nmol / gm tissue) and GSH (mmol / gm tissue) in aspirin induced gastric ulcer in rats (Mean ± SD).

Groups	NO	MDA	GSH
I	0.343± 0.02	43.94±1.653	3.31± 0.26
II	0.175±0.013•	57.97±1.416•	1.56± 0.1•
III	0.219±0.014*	53.00±1.333*	1.82± 0.16•
IV	0.408± 0.019*	44.38±1.738*	2.84± 0.18*
V	0.272± 0.016*	46.86 ± 1.517*	2.75± 0.21*
VI	0.22 ± 0.018*	51.18 ± 1.263*	2.57± 0.24*
VII	0.457±0.018•*#	44.01±1.07*#	3.31± 0.27*#
VIII	0.273±0.012•*#	46.52±0.756*#	3.18± 0.21*#
IX	0.239±0.023•*	49.72± 1.307*#	2.86± 0.17*#
P	<0.0001	<0.0001	<0.0001

• Significant difference in comparison to group I. * Significant difference in comparison to group II.
Significant difference in comparison to group III.

DISCUSSION

Non-steroidal anti-inflammatory drugs are widely used with major limitation is their potentially serious risk of gastrointestinal side effects ranging in severity from mild dyspepsia to gastrointestinal hemorrhage and perforation.[23]

In our study, macroscopic examination of gastric mucosa following administration of a single oral dose of aspirin to 24 hours fasted rats showed significant increase in gastric score lesion as compared to normal group. Previous studies considered that deficiency of PG plays a key role in NSAID induced gastrointestinal side effects. In the GI mucosa, the principal metabolic products of COX enzymes are the gastroprotective prostaglandins so that in the presence of a COX inhibitor, such as aspirin or other NSAIDs variable degrees of GI mucosal injuries occur.[24]

In the present study, aspirin caused a significant increase in the free and total gastric HCl levels and a significant decrease in the mucin level in the gastric content as compared to the normal control group. Previously, it was reported that endogenous PGs play a role in the regulation of various gastric functions, such as acid secretion,[25] mucus/ bicarbonate secretion, [26] and mucosal blood flow[27] that may contribute to gastric cytoprotection. Therefore, in the present study, the observed rise in free and total acidity and the decrease in the mucin level in gastric content as compared to normal control group can be attributed to depletion of mucosal PGs by inhibiting COX activity. Also a decrease in mucosal NO level following aspirin-induced injury was observed in the present study. PGE_2 depletion has been reported to decrease intracellular cAMP and trans-membrane uptake of L-arginine, the source of NO, from outside the cell.[28] NSAIDs lead also to leukocyte infiltration which is a major source of a superoxide radical anion that reacts with cellular lipids, forming lipid peroxides metabolized to MDA.[29] In agreement with this, depletion of GSH and rise MDA was observed in the present study indicating tissue oxidative stress. These findings coincide with the results of other studies.[30,31]

The present study demonstrated that three days prophylactic ranitidine before aspirin administration decreased the gastric lesion score significantly compared to the aspirin-injury group. Biochemical examination of gastric content showed significantly decreased level of HCl compared to aspirin-injury group. This is anticipated for ranitidine as a competitive inhibitor of histamine-2 receptors on the basement membrane of the parietal cells that inhibits the stimulatory effect of histamine released by enterochromaffin like cells as well as the effect of other substances that promote acid secretion.[32] The present study also showed that mucin level and NO level in the gastric tissue are significantly less compromised in the ranitidine-pretreated group compared to aspirin-injury group. In addition, the ranitidine treated group showed less rise in gastric tissue MDA content while GSH level was insignificantly different from that of the aspirin-injury group. Obviously, conservation of mucosal integrity to a significant degree by ranitidine administration compared to aspirin-injury group could be a leading factor in the upkeep of mucosal capacity to synthesize mucin and NO. Adding to this, Zhou et al[33] reported that ranitidine could induce up-regulation of the expression of mucin gene mRNA leading to an increase in gastric mucin secretion. Similarly, the mucosal protective effect of ranitidine explains its currently observed mild antioxidant effects which agree with the previously reported less rise in mucosal catalase and glutathione S-transferase activities accompanying ranitidine administration.[34]

The present study demonstrated that three days prophylactic administration of L-arginine was associated with a significantly lower gastric lesion score. Ohta and Nishida[35] suggested that exogenously administered L-arginine to rats subjected to stress-induced gastric mucosal lesions could cause significant gastroprotection and nitric oxide-mediated inhibition of neutrophil infiltration in gastric mucosal tissue. Also, the recent study by Heeba et al[36] reported that L-arginine augmented the gastroprotective effect of simvastatin in indomethacin-induced gastric ulcer in rats. NO donors have been reported to increase mucous thickness in rats' gastric mucosa.[37] Treatment with L-arginine was associated with less free and total HCl, increased mucin level in the gastric secretion and gastric mucosal NO compared to aspirin-injury group. In addition, significant increase in the level of GSH and significant decrease in the level of MDA were found in the L-arginine treated group compared to aspirin-injury group, indicating a significant antioxidant effect for L-arginine. The gastroprotective effect of L-arginine seen in the present study can be partly due to NO-mediated antioxidant effect as it was reported that the balance between NO and ROS levels, is important in the maintenance of proper endothelial function.[38] The availability of L-arginine decreases superoxide release and protects NO from inactivation by these superoxide anions.[39] L-arginine can also act as a scavenger for superoxide anion through its alpha-amino group and has been reported to protect against aspirin-induced mucosal damage, through a xanthine-xanthine oxidase antioxidant effect.[40, 41]

The present study tested the protective effect of three days treatment with zinc sulfate against aspirin-induced gastric injury. The study showed also in this zinc treated group, a significant decrease in gastric mucosal lesion score with significantly

lower gastric HCl and higher mucin levels compared to aspirin injury group. The level of gastric mucosal NO was significantly higher than aspirin treated group (but less than that of L-arginine treated groups). In addition, a significantly higher level of gastric GSH and lower level of MDA were detected compared to aspirin treated group. Ciesielska et al suggested two mechanisms to be responsible for the cellular protective effect of zinc: an antioxidant and antiapoptotic actions.[42] Another study attributed this effect to the ability of zinc to inhibit lipid peroxidation and to preserve mucosal NOS.[43] Zinc is also a cofactor for Cu- and Zn- superoxide dismutase (SOD),[44] a function that contributes to the cellular antioxidant capacity. Zinc deficiency is associated with lower SOD activity and a greater susceptibility to oxidative damage due to elevated peroxynitrite concentrations.[45] Zinc supplementation produced inhibition of H^+ back-diffusion and improvement of the microcirculation with membrane-stabilizing action on mast cells that decreases the histamine release and HCl secretion.[46] Also, it was reported that zinc can increase the mucin secretion in the large intestine of pigs.[47] In addition zinc was suggested to act as an additional prosthetic group of NOS, this can explain the increase in the gastric mucosal NO level in response to zinc treatment.[48]

In the present study, three days prophylactic oral treatment with pioglitazone was associated with significantly lower gastric lesion score, free and total acidity as well as higher gastric content of mucin as compared to aspirin injury group. This coincides with the previously reported gastroprotective effect of pioglitazone. Being a PPAR-γ ligand, pioglitazone is suggested to reduce in TNF-α and COX-2 expression and to up-regulate leptin mRNA in the gastric mucosa.[49] Examination of the gastric content revealed that the level of gastric HCl was significantly decreased and the level of mucin was significantly increased, in comparison to the aspirin treated group. Brzozowski et al[50] attributed the pioglitazone mediated gastroprotection to attenuation of expression and release of the pro-inflammatory cytokines TNF-α and IL-1β as well as to the enhancement of angiogenesis at ulcer margin. The same authors attributed the antisecretory effect of pioglitazone to its stimulant effect on the gastric mucosal PGE_2 synthesis. Significantly higher level of gastric NO level with significantly less oxidative stress in the pioglitazone treated group compared to aspirin injury group was also seen in our study. This antioxidant effect was reported by pioglitazone in previous studies.[51-53]

In relation to peptic ulcer, combined administration of an antisecretory drug with a cytoprotective agent is a frequent combination to provide a greater benefit. Accordingly the present study included prophylactic co-administration of ranitidine with either L-arginine, zinc sulfate or pioglitazone. The study showed that ranitidine/L-arginine is statistically the most effective preventive therapy against aspirin-induced mucosal injury assessed by gastric lesion score. In both the ranitidine/zinc sulfate and ranitidine/pioglitazone groups significantly lower gastric lesion scores were encountered compared to ranitidine monotherapy, yet a significant difference existed compared to normal control and the ranitidine/L-arginine groups. The addition of L-arginine to ranitidine was associated with a lower level of total acidity together with a higher level of mucin in gastric content and gastric tissue NO compared to ranitidine monotherapy group. A significantly lower level of total acidity was encountered in the ranitidine/zinc sulfate and ranitidine/pioglitazone groups compared to ranitidine monotherapy. On the other hand, a significantly higher mucin level in gastric content and the gastric tissue NO was found in the ranitidine /zinc sulfate group but not in ranitidine/pioglitazone group compared to ranitidine monotherapy. Again compared to ranitidine monotherapy, all three tested combinations were accompanied by significantly higher level of gastric tissue GSH and lower level of MDA. The antisecretory effect of ranitidine-zinc combination has been previously compared to ranitidine monotherapy in three experimental rat models of gastric ulceration and was found to show more gastroprotective against absolute ethanol and indomethacin than ranitidine monotherapy despite of a comparable antisecretory activity of ranitidine alone and in combination with zinc.[54,55] This distinct mucosal protection probably represents a synergistic approach against direct mucosal toxicity of acidic NSAID. Accordingly, the gastroprotective effect of ranitidine/zinc administration shown in the present study probably reflects the dual benefit of the antisecretory effect of ranitidine and the cytoprotective effect of zinc. Although the investigated parameters in the present study showed some favorable effect for this combination against acute aspirin-induced gastric injury, yet ranitidine /pioglitazone group was less protective than other studied combinations possibly because of the short duration of therapy as pioglitazone is a PPAR-γ agonist, that may need longer duration of administration to produce evident gastroprotective effect.

The clinically relevant translation of this area of research is that compounds that maintain mucosal blood flow are conceptual targets that might reduce GI injury due to NSAIDs. Some clinicians prefer proton pump inhibitors (PPIs) over H_2-blockers as prophylaxis against GI ulceration in patients at risk. However, the PPI-induced loss of normal stomach acidity and rise of gastric pH above six has been

associated with bacterial overgrowth of the upper GI tract. In addition, PPIs have also been shown to affect leukocytic function.[56] Because of these adverse effects and possibly others, it seems reasonable to try to optimize the benefits obtained from H_2-blockers in ulcer prophylaxis. **From the results of the present study it could be concluded that;** HCl, mucin, NO and oxidative stress are important factors involved in the pathogenesis of NSAID induced gastric ulcer, as evidenced by the protective effects of ranitidine, L-arginine, zinc sulfate and pioglitazone in aspirin induced gastric ulcer. The role of HCl and NO seems to be more important in the pathogenesis of aspirin induced gastric ulcer, as evidenced by the better protective effects of combination of ranitidine and L-arginine in comparison to the ranitidine with either zinc sulfate or pioglitazone.

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