Captopril versus enalapril in the protection of the gastric mucosa against NSAID induced gastric mucosal injury in rats

Samir Y. N. Matloub* **MBChB, MSC -DM**

Summary:

Background: Different mechanisms have been suggested for the development of nonsteroidal anti-inflammatory drugs (NSAIDs) induced gastropathy. Angiotensin converting enzyme inhibitors have been suggested to have gastroprotective effects. This study investigates the gastroprotective effects of Angiotensin converting enzyme inhibitors, captopril and enalapril on indomethacin induced gastric mucosal damage in rats .

Materials and methods: The study was conducted on 50 adult male albino rats, divided into 5 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 captopril or enalapril. In order to study the possible role of nitric oxide (NO) in the gastroprotective effect of captopril; intraperitoneal N^G-L-Arginine Methyl Ester (L-NAME) a nitric oxide synthase inhibitor was given 30 minutes prior to captopril administration followed by indomethacin and this served as fifth group.

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 prostaglandinE2 (PGE2), and myeloperoxidase (MPO).

Results: Captopril in a dose of 15 mg/kg produced a significant reduction ($p < 0.05$) in the gastric damage score .These protective effects were associated with a significant increase ($p < 0.05$) in gastric PGE2 levels and marked decrease ($p < 0.05$) in MPO activity, L-NAME pretreatment didn't abrogate the effects of captopril. Enalapril pretreatment failed to show the gastroprotective effects of captopril.

Conclusions: The prophylactic use of captopril in this study prevented indomethacin induced gastropathy .This protective effect was associated with PGE2 upregulation and decreased oxyradical generation reflected by a decrease in MPO activity .Enalapril failed to produce the gastroprotective effects of captopril.

Fac Med Baghdad 2011; Vol. 53, No. 2
Received Nov. 2010
Accepted Feb. 2011

Key words: Cytoprotection , **C**aptopril ,Enalapril , NSAID gastropathy

Introduction:

The pathogenesis of NSAIDs induced gastric mucosal injury is generally ascribed to inhibition of prostaglandins (PGs) synthesis (1). However , other mechanisms are also involved including the role of nitric oxide (NO)(2),oxygen derived free radicals generation(3), neutrophil adherence to the vascular endothelium(4), and cytokine expression (5) . It has been suggested that angiotensin converting enzyme inhibitors (I. ACE) could exert gastroprotective effects by enhancing endogenous prostaglandins and activation of the renin – kallikrein – kinin system resulting in NO formation(6) , which is an important reparative component of the gastric mucosa(7). Moreover it has been observed that thiol (SH) containing compounds may have the ability to exert gastric cytoprotective effects through increasing non-protein SH levels (8, 9).In this study the gastroprotective role of two different I. ACE namely captopril a short acting sulfhyryl containing drug and enalapril a prodrug against indomethacin induced gastropathy was investigated and their effects on PGE2 production, NO release and MPO activity were evaluated.

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

Materials and methods:

This study was conducted on 50 adult male albino-Wister rats weighing (200-250 g) .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) . Captopril and enalapril were dissolved each in the vehicle and their concentration was adjusted to 3.75 mg/ml and 1.25 mg/ml respectively. 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) (10). All drugs were freshly prepared immediately before use. The animals were divided into five groups of ten. 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 either captopril

15mg/kg or enalapril 5mg/kg. In order to study the role of NO in the protective effect of captopril, intraperitoneal L-NAME 20mg/kg was administered 30 minutes before captopril and served as the fifth group.

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^{o}C for biological assay .Assessment of gastric mucosal damage: Gastric damage score (GDS) was calculated by the summation of the lengths of all linear erosions according to Santucci ,*et al.* (1994)(11). 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^{o}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)(12). 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^{o}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^{o}C. The results were reported as the MPO unit /mg of tissue according to Bradley, *et al.* (1982) (13).

Statistical analysis:

Statistical analyses and graphics were performed using SPSS Ver. 13 software for Windows (Statistical Analysis for Social Sciences, Apache Software Foundation, and USA). 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. The level of statistical significance was set as *p value* < 0.05.

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 was observed 4 hrs after indomethacin administration. Indomethacin caused a significant ($p< 0.05$) mucosal injury represented by a gastric damage score of (35.71 $\pm$ 1.03mm) when compared with control group as shown in Figure (1). In addition indomethacin caused significant suppression ($p<0.05$) of gastric PGE2 mean (67 $\pm$ 1.53ng/g) versus (232 $\pm$ 4.78ng/g) in the control group as shown in figure (2). Also there was significant increase ($p<0.05$) in gastric MPO activity mean (29.4 $\pm$ 0.62u/mg) versus (4.74$\pm$0.13u/mg) in the control group as shown in figure (3). Captopril pretreated group: Captopril pretreatment caused significant reduction ($p< 0.05$) of GDS mean (0.5 $\pm$ 0.03mm) compared to (35.71 $\pm$ 1.03mm) in the indomethacin treated group as shown in figure (1) . Gastric PGE2 level was significantly increased ($p< 0.05$) mean **(** 134.2 $\pm$ 1.56ng/g**)** versus (67 $\pm$ 1.53ng/g) in the indomethacin treated group as shown in figure (2) .By evaluating the effect of captopril on MPO activity ; there was significant decrease ($p< 0.05$) in MPO activity mean (7.35 $\pm$ 0.31u/mg) compared to (29.4 $\pm$ 0.62u/mg) in the indomethacin treated group as shown in figure (3) . L-NAME pretreatment failed to reverse the gastroprotective effect of captopril GDS (0.55$\pm$ 0.02mm) compared to (0.5 $\pm$ 0.03 mm) in the captopril alone treated group as depicted in figure (1). Enalapril pretreated group: enalapril pretreatment failed to demonstrate gastroprotective action against indomethacin induced gastropathy ; GDS (32.10$\pm$ 0.93mm) compared to (35.71 $\pm$ 1.03mm) in the indomethacin treated group as shown in figure (1).This failure was correlated with the inability of enalapril to up regulates gastric PGE2 level mean **(** 77.1 $\pm$ 0.64ng/g **)** versus (67$\pm$ 1.53ng/g) in the indomethacin treated group as shown in figure (2) . in addition enalapril didn't demonstrate significant change in gastric MPO activity mean (26.38 $\pm$ 0.86u/mg) compared to (29.4$\pm$ 0.62u/mg) in the indomethacin treated group as shown in figure(3).

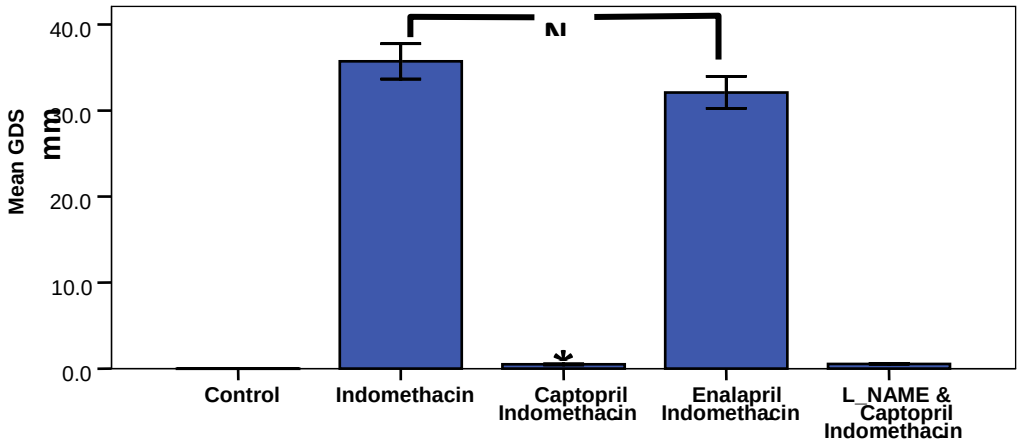

Figure (1): The effect of captopril versus enalapril pretreatment on the gastric damage score induced by indomethacin and the effect of L-NAME. The results are expressed as the mean ± SEM
*** P < 0.05 when compared with indomethacin group.**
NS: no significant

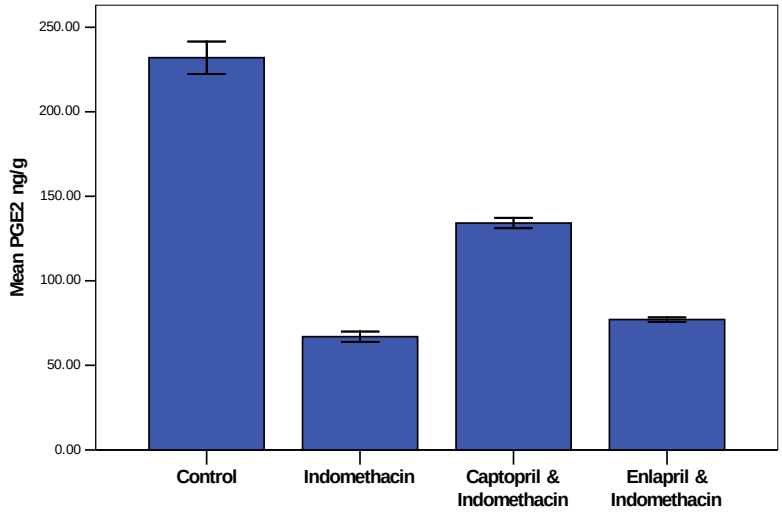

Fig. (2): The effect of captopril versus enalapril pretreatment on the gastric PGE2 levels inhibited by indomethacin. The results are expressed as the mean ± SEM.
***P< 0.05 when compared with control group.**
**** P < 0.05 when compared with indomethacin.**
NS: no significant

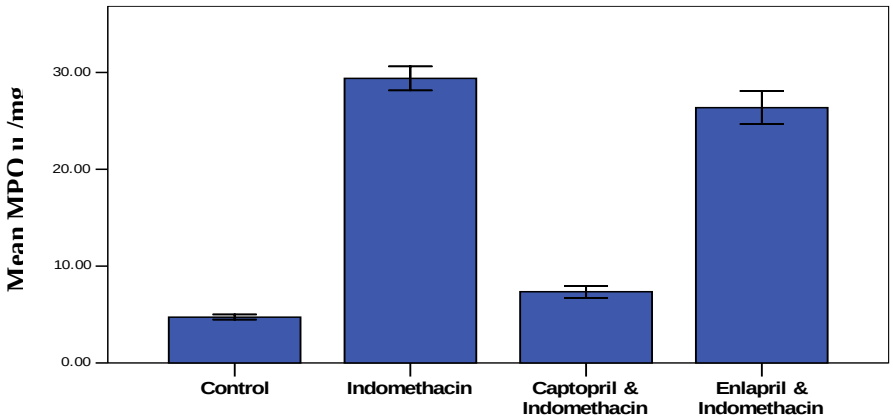

Fig.(3) : The effect of captopril versus enalapril pretreatment on the increased gastric MPO activity induced by indomethacin .The results are expressed as the mean ± SEM .
*** P < 0.05 when compared with control group.**
**** P < 0.05 when compared with indomethacin.**
NS: no significant

Discussion:

In this study pretreatment with 15mg/kg captopril elicited a significant decrease in the extent of the gastric damage caused by indomethacin. This protective effect was associated with upregulation of gastric PGE2 levels. This increase in PGE2 levels is in accordance with Gunal *et al* observations (14). It also seems likely that the free radical scavenging property of the sulfhydryl moiety of captopril adds to its protective effect (15). This was reflected in this study by the significant inhibition of MPO activity, a specific marker for oxyradical generation and neutrophil infiltration in tissues which are the early events of gastric damage associated with the use of NSAIDs (16, 17). On the other hand this study failed to demonstrate any role of NO in the protective mechanisms of captopril; this is because the administration of the nitric oxide synthase inhibitor L-NAME could not abrogate the protective effects of this drug. Although the general mechanism of enalapril is similar to that of captopril (18), however enalapril in this study failed to ameliorate the gastric damage neither neither inflicted by indomethacin nor influenced the MPO activity levels. Moreover enalapril caused no PGE2 up regulation. Other studies done on plasma PGE2, and renal PGE2, were also unable to show associated PGs changes with enalapril treatment (19, 20). It can also be concluded that enalapril had no effect on nitric oxide production since it has not exhibited any mucosal protective effects. The differences observed between captopril and enalapril in their ability to protect the gastric mucosa in this experiment could be partly explained on the basis of the pharmacokinetic profile of these two ACE inhibitors, where captopril is active by itself and has a rapid onset of action while enalapril is a prodrug with a delayed onset of action that requires after absorption de-estrification by the liver to the active enalaprilat (21). Therefore the time which was allowed for enalapril to produce its effects in this study was rather short.Moreover the lack of the SH moiety in the structure of enalapril could also have contributed to the loss of its gastroprotective effects and its effects on the MPO activity levels. The behavior of the other members of the ACE inhibitors family regarding gastric protection remain to be tested in future studies.

References:

(1) Kathleen M Knights, Arduino A Mangoni, and John O Miners . Defining the COX Inhibitor Selectivity of NSAIDs: Implications for Understanding Toxicity. Expert Rev Clin Pharmacol. 2010;3(6):769-776.

(2) Li Ma and John L Wallace . Endothelial nitric oxide synthase modulates gastric ulcer healing in rats. American . Journal .Physiol. Gastrointestinal. Liver Physiol. 2000 ;279: G341-G346.

(3) Santos CL, Marcellus H.L.P, Antoniella S Gomes, Henrique Lemos P, and Wallace L .Sildenafil prevents indomethacin induced gastropathy in rats: British .Journal . Pharmacol. 2005 ;146: 481-486.

(4) Outi Kosonen , Hannu Kankaanranta , Ulla Malo-Ranta and Eeva Moilanen . Nitric oxide-releasing compounds inhibit neutrophil adhesion to endothelial cells. European Journal . Pharmacol . 1999; 382: 111-117 **.**

(5) Mascagni . P, Sabbatini . V, Biordi. L, et al : R and R isomers of NSAIDs differentially regulate cytokine production. European Cytokine Network . 2000 ; 11(2) : 185-192.

(6) Alvin H. Schmaier . The kallikrein-kinin and the renin-angiotensin systems have a multilayered interaction. American journal of physiology - Regu. Physiol . 2003 ; 285 (1 **)** *: R1-R13 .*

(7) Iakubov AV, and Usmanova Sh E. Prophylactic use of angiotensin-converting enzyme inhibitors in indomethacin-induced ulcer and erosion lesions of the stomach . Likars'ka sprava / Ministerstvo okhorony zdorov'ia Ukraïny. 2004 ;47(9):1019-5297.

(8) Toyokuni S . Reactive oxygen species – induced molecular damage and it is applications in pathology. Pathol. Int. 1999; 49: 91-102.

(9) Salim AS . Protection against chronic ulceration in rat with sulfhydryl containing agents . Journal Pharm. Sci , 1992; 81 : 698-700.

(10) Griffith O.W, and Kilbourn R.G. Nitric oxide synthase inhibitors , amino acids. Methods .Journal . enzymol. 1996; 268 :375-392.

(11) Santucci L, Fiorucci S. and Giansanti M. Pentoxifylline prevents indomethacin-induced acute gastric mucosal damage in rats. Role of TNF-α, Gut. 1994; 35: 909-915.

(12) 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. Journal. Gastroenterol. 2000; 119:706-714.

(13) Bradley P.P, Christensen R.D and Rothstein G. Cellular and extracellular myeloperoxidase in pyogenic inflammation, Blood. 1982 ;60: 618-622.

(14) Günal Ö , Özdemir Aktan ' A , Yegen C , Kurtel H and Yalin R. Captopril prevents the oxidative damage to proteins after renal ischemia reperfusion injury: role of endothelin-1. Prostaglandins, Leukotrienes and Essential Fatty Acids. 1997; 56(1): 23-27.

(15) Mario Barbagallo; Ligia J. Dominguez; and Lawrence M. Resnick . Protective Effects of Captopril Against Ischemic Stress .Role of Cellular Mg. Hypertension. *1999;34:958-963 .*

(16) Bayir Y , Odabasoglu F , Cakir A , et al . The inhibition of gastric mucosal lesion , oxidative stress and neutrophil infiltration in rats by lichen constituent diffractaic acid . phytomedicine. 2006;13: 584- 590.

(17) Odabasoglu F, Cakir A , Suleyman H, Aslan A , Bayir Y Halici M and Kazaz C. Gastroprotective and antioxidant effects of usnic acid on indomethacin induced gastric ulcer n rats . Journal Ethnopharmacol.2006; 1: 59- 65.

(18) Brian B. Hoffman .Therapy of hypertension. In: Goodman and Gilman's the pharmacological basis of therapeutics. (Laurence L Burton. John S Lazo and Keith L. Parker.)section V, ch:32. McGraw Hill. 2006 : 858-860.

(19) Sunaga K., Fujimura A., Shiga T. and Ebihara A. Chronopharmacology of enalapril in hypertensive patients . European Journal of Clinical Pharmacol. 1995;48(6): 441-445.

(20) Stephen L. Swartz . The role of prostaglandins in mediating the effects of angiotensin converting enzyme inhibitors and other antihypertensive drugs. Cardiovascular Drugs and Therapy. 1997; 1 (1) :39-43.

(21) Edwin K .Jackson .Renin and angiotensin In: Goodman and Gilman's the pharmacological basis of therapeutics. (Laurence L Brunton. John S Lazo and Keith L. Parker.) 2006 , section V , ch:30. McGraw Hill : 802-803.