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J. Sci. Trans. Environ. Technov. 2011, 4(4) :151-154

Scientific Transactions in Environment and Technovation

A comparative study of Ranitidine and Esomeprazole on nitric oxide production in indomethacin induced ulcerated rats

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Abstract

The present study was designed to evaluate the effect of Esomeprazole and Ranitidine on Nitric oxide production in Indomethacin induced ulcerated rats.Indomethacin induce gastric damage by decreasing the production of nitric oxide and prostaglandins,which was reversed by Esomeprazole and Ranitidine.The results also suggest that the former is more effective than the latter in restoring NO production thereby protecting the gastric lumen.

Keywords: esomeprazole, indomethacin, nitric oxide, peptic ulcer, ranitidine

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have become one of the most commonly used medications.NSAIDs are capable of inducing widespread injury throughout gastro intestinal tract and liver (Lanas *et al.*, 1992). NSAIDs have been used since the beginning of the last century (Tegeder *et al.*, 2001). However,these drugs have direct side effects on the gastro intestinal tract ranging from dyspepsia and abdominal pain to bleeding and perforated ulcers (Kokoska *et al.*, 1998). The relationship between NSAIDs and the complication of peptic ulcer disease are now well established (Cryer and Feldman, 1992). Fully 20-25% of all patients chronically taking NSAIDs will develop gastric or duodenal ulcers (Larkai *et al.*, 1987 and Bellary *et al.*, 1991). It has been well known that NSAIDs such as indomethacin produce gastro intestinal injury in both humans (Katz *et al.*, 1965 and Davies & Wallace, 1997) and animals (Coli *et al.*, 1967, Djahanguiri, 1969 and Kent *et al.*, 1969).

NO produced in the gastric lumen after nitrate ingestion increases gastric mucosal blood flow and the thickness of the firmly adherent mucous layer in the stomach.The blood flow and the mucous layer are essential defence mechanisms,which protect the mucosa from luminal acid and noxious agents.NSAIDs induce gastric damage by decreasing NO production and prostaglandin.But NO has a protective role in stomach, by healing intestinal lesions, and enhancing the prostaglandin production.NO acts as antioxidant, thereby protecting gastric lumen against indomethacin-induced ulceration (Petersson, 2008).

Antacids will reduce the ulcerogenecity of NSAIDs (Scheiman, 1999). Ranitidine belongs to a group of drugs called histamine-2 blockers. It is used to treat and prevent ulcers in the stomach and small intestine. Esomeprazole reduces gastric acid secretion through inhibition of H^+ / K^+ ATPase in gastric parietal cells. By inhibiting the functioning of this enzyme, the drug prevents formation of gastric acid (Lind *et al.*, 2000). Ranitidine and Omeprazole were given as medicines for the treatment of NSAID associated ulcers. The gastro protective effect of Esomeprazole and Ranitidine in ulcerated rats has been evaluated through various biochemical parameters and presented in this paper.

MATERIALS

Animals

Adult male rats weighing about 120-150g were kept under controlled laboratory conditions throughout the experimental work. The animals were purchased from the animal house, Mayavaram. Experimental procedures were adopted as approved by the animal experimentation ethical committee.

Chemicals

Drugs such as Indomethacin, Ranitidine and Esomeprazole used in the study were purchased from Micro Labs Ltd, Torrent pharmaceuticals Ltd, and Glen mark Pvt Ltd respectively.

METHODS

Experimental design

Animals were divided into four groups of six each.

Group 1 : Rats served as control, administered with saline (120±10, orally).

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Group 2 : Rats were administered with indomethacin (20 mg/ kg b.w., 130±10).

Group 3 : Rats were administered with Ranitidine (150 mg/ kg b.w., subcutaneously) along with indomethacin (20 mg / kg b.w., 150±10,orally).

Group 4 : Rats were administered with Esomeprazole (20 mg / kg b.w., subcutaneously) along with indomethacin (20 mg / kg b.w., 160±10,orally).

After stipulated time, the rats were sacrificed. Serum, plasma and tissue supernatant were used for biochemical investigations using standard procedures.

RESULTS AND DISCUSSION

Peptic ulcer disease occurs mainly due to conception of NSAIDs, infection by *Helicobacter pylori*, stress or due to pathological conditions such as zollinger-ellison syndrome. The pathophysiology of experimental peptic ulcer formation is believed to be multifactorial (Guzel *et al*., 1998; Dhikav *et al*., 2003). Cause of PUD due to NSAIDs include increase in acid secretion, reduction of gastric mucosal blood flow, inhibition of prostaglandin synthesis, disruption of mucosal barrier, inhibition of mucus and bicarbonate secretion in the gastro intestinal mucosa (Allen and Leonard 1988; Aase 1989), that is an imbalance between increased aggressive factors and decreased protective factors. (Sarkar *et al*., 2006).

Parameters such as gastric pH, gastric juice volume, mucosal proteins, Hb, RBC, WBC, PCV (Table 1), Liver marker enzymes, serum electrolytes (Table 2) have been analysed. The results suggest that indomethacin alters the parameters to considerable extent, which were restored to near normal with Ranitidine and Esomeprazole. Further, Esomeprazole was found to be more potent in restoring normalcy than Ranitidine.

NSAIDs induce ulcer mainly due to inhibition of prostaglandin (Barnett *et al*., 2000). Prostaglandin derived from COX I has inhibitory effect on hydrochloric acid secretion in the stomach cells. NSAIDs block the synthesis of prostaglandin by inhibiting cyclooxygenase (COX I and COX II). Inhibition of both COX induces gastric ulcer. (Chakraborty *et al*., 1996). Higher does of indomethacin can cause disruption of oxidative phosphorylation and inhibit the biosynthesis of mucus polysaccharides (Ment *et al*., 1994).

Indomethacin induces H^+ / K^+ ATPase in gastric parietal cells. Thus increases the gastric acid secretion whereas anti ulcer drugs inhibit H^+ / K^+ ATPase in gastric parietal cells. In the present study, the level of gastric volume was significantly elevated (+102.17%) and the pH was significantly decreased (- 41.19%) in ulcer induced rats. Administration of antiulcer drugs (Ranitidine and Esomeprazole) significantly reduced the gastric juice volume (-31.18%,-25.80%) increased the pH (+17.83%, +29.29%) when compared to ulcer-induced rats.

The gastric mucosal protein is highly decreased (-43.24%) in ulcerated rats which was significantly increased by antiulcer drugs (23.80%, +42.85%). Decrease in Hb, RBC, PCV might be due to internal bleeding and perforation while increase in WBC might be due to inflammatory action of defense mechanism. There was a significant increase in the level of liver marker enzymes like ALT (+172.22%), AST (+ 117.10), ALP (+14.39%) and LDH (+326.15%) in ulcer induced rats. Treatment with anti ulcer drugs brought back the parameters to normal.

Table : 1 Effects of Ranitidine and Esomeprazole on the garstric and hematological parameters in ulcer induced rats.

Groups	Gastric Profile			Hematological Profile			
	pH	Volume (µl)	Mucosal Protein (g/dL)	Hb (g/dL)	WBC (TC) (Cells / Cubic mm)	RBC (Millions/ Cubic mm)	PCV (%)
Group I (Control)	2.67±0.15	2.3±0.16	1.85±0.12	15.32±0.78	4700±147.19	4.3±0.34	42±4.89
Group II (Indomethacin)	1.57±0.09 (-41.19%)	4.65±0.96 (102.17%)	1.05±0.12 (-43.24%)	7.92±0.97 (-48.30%)	7150±631.13 (+52.12%)	2.9±0.18 -32.55%)	26±2.44 (-38.09%)
Group III (Indomethacin + Ranitidine)	1.85±0.04 (+17.83%)	3.2±0.89 (-31.18%)	1.3±0.08 (+23.80%)	10.52±0.36 (+32.82%)	5858±339.11 (+18.18%)	3.75±0.20 (+29.31%)	30±3.74 (+15.38%)
Group IV (Indomethacin + Esomeprazole)	2.03±.0.01 (+29.29%)	3.45±1.40 (-25.80%)	1.5±0.08 (+42.85%)	12.35±0.36 (+55.93%)	4975±379.69 (+30.41%)	4.2±0.21 (+44.82%)	38±3.16 (+46.15%)

Values are mean ±S.E (n=6). Statistical Comparison of Group II Vs Group I, Group III & IV Vs Group II. Values in parentheses show percent increase (+) or decrease (-) over group I & II.

Table : 2 Effects of Ranitidine and Esomeprazole on the Liver marker enzymes and serum electrolytes in ulcer induced rats.

Groups	Liver Marker enzymes				Serum electrolytes		
	AST U/L	ALT U/L	LDH U/L	ALP (U/L)	Sodium meq /L	Potassium meq/L	Calcium mg/dL
Group I (Control)	76±8.20	27±9.62	130±12.24	133.75±3.5	146.75±2.5	4.72±0.43	9.7±0.46
Group II (Indomethacin)	165±14.71 (+117.10%)	73.5±8.53 (+172.22%)	554±23.9 (+326.15%)	153±3.46 (+14.39%)	154.5±3.10 (+5.28%)	5.17±+0.33 (+9.53%)	10.07±0.05 (+3.81%)
Group III (Indomethacin + Ranitidine)	103±15.89 (-37.57%)	50±9.12 (-33.97%)	372±26.08 (-32.85%)	129±4.76 (-15.68%)	150.25±9.17 (-2.75%)	4.95±0.33 (-4.25%)	9.95±0.1 (-1.19%)
Group IV (Indomethacin + Esomeprazole)	80±12.90 (-15.51%)	32.5±6.45 (-55.78%)	325±45.6 (-41.33%)	135±2.44 (-11.76%)	149.25±2.98 (-3.39%)	4.7±.0.14 (-9.09%)	9.85±0.23 (-2.18%)

Values are mean ±S.E (n=6). Statistical Comparison of Group II Vs Group I, Group III & IV Vs Group II. Values in parentheses show percent increase (+) or decrease (-) over group I & II.

Indomethacin also reduces plasma renin activity, aldosterone level and increases sodium and potassium retention. It also enhances the effect of vasopressin, which leads to oedema, hyperkalemia, hypernatremia and hypertension (Akbarpour *et al.*, 1985). In the same line, significant alterations were found in the levels of serum electrolytes.

Reactive oxygen species (ROS) have also shown to play a critical role in the development of acute experimental gastric lesions induced by NSAIDs (Das *et al.*, 1997). ROS damage membrane proteins by causing lipid peroxidation is measured as the amount of TBARS in the gastric mucosa. The present study also correlates with these findings. There was a significant elevation (+32.80%) of TBARS in the gastric mucosa in ulcerated group of rats. Treatment with anti ulcer drugs significantly reduced (-24.50%, -31.12%) the level of TBARS (Table 3).

Experimental evidence indicates that an imbalance in the production and removal of ROS play a crucial role in gastric mucosal damages due to indomethacin and other NSAIDs (Halici *et al.,2005;* Elliot and Wallace, 1998; Basiviredy *et a*l., 2003; Bayir et al., 2006). Indomethacin performs pro-oxidant activity, initiates lipid peroxidation and decreases SOD activity by generating ROS, thereby interfering with the mucosal cells endogenous antioxidant-systems (Yoshikawa *et al.*, 1993; Takeuchi *et al.*, 1991; Naito *et al.*, 1998). As a consequence of this process, oxidative damage occurs (Figge and Figge,1990; Sedlak and Lindsay, 1968). Organisms do, however, have enzymatic and nonenzymatic defense mechanisms against the toxicity and tissue damage of ROS (Bradley *et al.*,1982). It has been reported that superoxide dismutase activity in rat stomach tissues is decreased by NSAIDs. It destroys the highly reactive radical O_2 by converting it into the

Table : 3 Effects of Ranitidine and Esomeprazole on NO, TBARS, SOD in ulcer induced rats

Groups	NO		TBARS mmol/100g tissue	SOD U/mg tissue
	Serum µmol/L	Tissue µmol/mg		
Group I (Control)	50±9.12	467.5±19.36	1.137±0.08	10.65±0.89
Group II (Indomethacin)	82.5±6.45 (+65%)	292.5±25.98 (-37.43%)	1.51±0.06 (+32.80%)	7.62±0.63 (-28.45%)
Group III (Indomethacin + Ranitidine)	70.75±7.88 (-14.24%)	360±19.57 (+23.07%)	1.14±0.08 (-24.50%)	8.82±0.22 (+15.74%)
Group IV (Indomethacin + Esomeprazole)	68.5±5.80 (-16.96%)	388.75±8.53 (+32.90%)	1.04±0.05 (+31.12%)	9.05±0.12 (+18.76%)

Values are mean ±S.E (n=6). Statistical Comparison of Group II Vs Group I, Group III & IV Vs Group II. Values in parentheses show percent increase (+) or decrease (-) over group I & II.

less reactive H_2O_2 that can be destroyed by the catalase reaction.

The activity of SOD in gastric mucosa was significantly decreased (-28.45%) in ulcerated rats when compared to normal group. On treatment with ranitidine (+15.74%) and Esomeprazole (+18.76%), the level of SOD is increased and its activity is restored. Thus the present results agree with the previous findings.

Indomethacin-induced gastric injury mediated by the reduction in tissue cNOS-derived NO content and enhanced production of iNOS-derived NO in serum. Our findings concerning NO are in agreement with the widely accepted fact that, in the digestive system, NO produced by cNOS is cytoprotective and NO produced by iNOS is cytotoxic (Nishida *et al*., 1998). The present study also correlates in the above findings, it could be concluded that increased acid production decreased pH, mucosal protein, alteration in NO, oxidative stress, decreased SOD are contributing to indomethacin induced gastric ulceration. It was also demonstrated that administration of H_2 blocker (Ranitidine) or PPI (Esomeprazole) has gastroprotective potential and the present findings also suggest that Esomeprazole is more potent than Ranitidine in healing.

REFERENCES

Aase, S. 1989. Disturbances in the balance between aggressive and protective factors in the gastric and duodenal mucosa. *Scand.J.Gastroenterol*., 24: 17.

Akbarpour, F., Afrasiabi, A. and Vaziri, N. 1985. Severe hyperkalemia caused by indomethacin and potassium supplementation. *South Med.J.*, 78:756-757.

Allen, A. and Leonarn, J.A. 1985. The mucus barrier: Its role in gastroduodenal mucosal protection.*J.Clin. Gastroenterol*., 10:593.

Barnett, K., Bell, C.J. and McKnight, W. 2000. Role of cyclooxygenase-2 in modulating gastric acid secretion in the normal and ulcerated rat stomach. *Am. J. Physiol. Gastrointest. Liver Physiol*., 279: G1292-G1297.

Basiviredy, J., Jacob, M., Ramamoorthy, P., Pulimood, A.B. and Balasubramanian, K.A. 2003. Indomethacin induced free radical mediated changes in the intestinal brush border membranes. *Biochem. Pharmacol*., 65: 683-695.

Bayir, Y., Odabasoglu, F., Lakir, A., Aslan, A., Suleyman, H. and Halici, M. 2006. The inhibition of gastric mucosal lesion oxidative stress and neutrophil-infiltration in rats by the lichen constituent diffractaic acid. *Phytomedicine.*,13: 584-590.

Bellary, S.V., Isaacs, P.E.T. and Lee, F.I. 1991. Upper gastrointestinal lesions in elderly patients presenting for endoscopy: relevance of NSAID usage. *Am.J.Gastroenterol*., 89: 961-964.

Bradley, P.P., Priebat, D.A., Chriestensen. R.D. and Rothstein. G. 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J.Invest.Dermatol.*,78: 206-209.

Chakraborty, I., Das, S.K., Wang., et al. 1996. Developmental expression of the cox-1 and cox-2 genes in the peri-implantation mouse uterus and this differential regulation by the blastocyst and ovarian steroids.*J. Mol. Endocrinol*.,16: 107-122.

Coli, V., Silvestrini, B. and Dordoni, F. 1967. Evaluation of the potential of gastric ulceration after administration of certain drugs. *Exp.Mol.Pathol*., 6:68-83.

Cryer, B. and Feldman, M. 1992. Effects of NSAIDs on endogenous gastrointestinal prostaglandins and therapeutic strategies for prevention and treatment of NSAID - induced damage. *Arch. Intern.Med*., 152:1145-1155.

Das, D., Bandyopadhyay, D., Bhattacharjee, M. and Banerjee, R.K. 1997. Hydroxyl radical is the major causative factor in stress-induced gastric ulceration. *Free Radical.Biol. Med*., 23: 8-18.

Davies, N. M. and Wallace, J. L. 1997. NSAID-induced gastrointestinal toxicity: new insights into an old problem. *J.Gastroenterol*., 32: 127-133.

Dhikav, V., Singh, S., Pande, S., Chawla, A. and Singh, A.K. 2003. Non-Steroidal drug-induced gastrointestinal toxicity: Mechanisms and management. *J. Indian Acade.Clin.Med*., 4: 315.

Djahanguiri, B. 1969. The production of acute gastric ulceration by indomethacin in the rats. *Scand. J.Gastroenterol*., 4: 265-267.

Elliot., S.N. and Wallace, J.L. 1998. Nitric oxide: a regulator of mucosal defense and injury. *J.Gastrenterol*., 33: 792-803.

Figge, H.L. and Figge, J. 1990. The effects of amiodarone on thyroid hormone function: A review of the physiology and clinical manifestations. *J. Clin. Pharmacol*., 30: 588-595.

Guzel, C., Kurt, D., Sermet, A., Zeki, K., Denli, O. and Canoruc, F. 1998. The effects of vitamin E on gastric ulcers and gastric mucosal barrier in stress induced rats. *Tr.J.Med.Sci*., 28: 19.

Halici, M., Odabasoglu, F., Suleyman, H., Cakir, A., Aslan, A. and Bayir, Y. 2005. Effects of water extract of *Usnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. *Phytomedicine*, 12: 655-662.

Katz, A.M., Pearson, C.M. and Kennedy, J.N. 1965. A clinical trial of indomethacin in rheumatoid arthritis. *Clin. Pharmacol.Ther.*, 6: 25-30.

Kent, T.K., Cardelli, R.M. and Stamler, F.W. 1969. Small intestinal ulcers and intestinal flora in rats given indomethacin. *Am.J.Pathol.*, 54: 237-249.

Kokoska, E.R., Smith, J.S., and Deshpande, Y. 1998. Indomethacin increases susceptibility to injury in human gastric cells independent of prostaglandin synthesis inhibition. *Am.J.Physiol.Gastrointest.Liver Physiol.*, 275: G620-G628.

Lanas, A., Sekar, M.C. and Hirshowitz, B.I. 1992. Objective evidence of aspirin use in both ulcer and non-ulcer upper and lower gastrointestinal bleeding. *Gastroenterology*, 103: 862-869.

Larkai, E.N., Smith. J.L., Lidskey, M.D., and Graham, D.Y. 1987. Gastroduodenal mucosa and dyspeptic symptoms in arthritic patients during chronic NSAID use. *Am.J.Gastroenterol.*, 1153-1158.

Lind, T., Rydberg, L., Kyleback, A., Jonsson, A., Andersson, T., Hasseigren, T., Holmberg, J. and Rohss, K. 2000. Esomeprazole provides improved acid control vs. omeprazole in patients with symptoms of gastro-esophageal reflux disease. *Alimentary pharmacology & therapeutics.*, 14: 861-867.

Ment, I.R., Oh, W. Ehrenkrantz, R.A., et al. 1994. Low dose indomethacin and prevention of intraventricular hemorrhage multi-center randomized trial. *Pediatrics.*, 93: 543-550.

Naito, Y., Yoshikawa, T., Yoshida, N. and Kondo, M. 1998. Role of oxygen radical and lipid peroxidation in indomethacin-induced gastric mucosal injury. *Dig. Dis. Sci.*, 43: 30S-34S.

Nishida, K., Ohta, Y. and Ishiguro, I. 1998. Relationship between constitutive nitric oxide synthase activity and mucus level in the gastric mucosa of rats with stress. *Pharmacol.Res.*, 38: 393-400.

Petersson, J. 2008. Nitrate, Nitrite and Nitric oxide in Gastric mucosal defense. Acta Universitatis Upsaliensis. *Digital comprehensive summaries of Uppsala Dissertations from the faculty of Medicine.* 328: 90.

Sarkar, N., Purkayashtha, S., Sarkar, B. and Guha, D. 2006. Modulation of gastric mast cell population: Role of vestibulo cerebellar lesion. *Indian J.Exp.Biol.*, 44:627.

Scheiman, J.M. 1999. Preventing NSAID toxicity to the upper gastrointestinal tract. *Gastroenterology*, 2: 205-213.

Sedlak, J. and Lindsay, R.H. 1968. Estimation of total, protein-bound and non protein sulfhydryls groups in tissue with Ellman's reagent. *Anal.Biochem.*, 25:192-205.

Takeuchi, K. and Okabes, S. 1991. Effect of indomethacin on gastric mucosal blood flow around acetic acid-induced gastric ulcer in rats. *Gastroenterology*, 100:1259-1265.

Tegeder, I., Pfelisehifter, I., and Geisslinger, G. 2001. Cyclooxygenase independent actions of cyclooxygenase inhibitors. *FASEBJ*, 15: 2057-2072.

Yoshikawa, T., Naito, Y., Kishi, A., Tomii, T., Kaneto, T., Linuma, S., Ichikawa, H., Yasuda, M., Takahashi, S. and Kondo, M. 1993. Role of active oxygen, lipid peroxidation and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. *Gut.*, 34: 732-737.