Indian J Physiol Pharmacol 1999; 44 (2) : 185–191

EFFECT OF MILD IRRITANT ON GASTRIC MUCOSAL OFFENSIVE AND DEFENSIVE FACTORS

CH. V. RAO. R. N. MAITI AND R. K. GOEL*

Department of Pharmacology ,
Intstitute of Medical Sciences,
B. H. U., Varanasi – 221 005

(Received on September 7, 1999)

Abstract : The effect of hypotonic medium (Distilled water : DW) and hypertonic saline (HS :5% NaCl) compared to control normal saline (NS) was studied on gastric ulcer induced by aspirin, 6 h cold restraint stress, ethanol, and pylorus ligation in rats. DW did not afford any protection while HS showed significant ulcer protective effects in all gastric ulcer models studied. The cytoprotective effect of HS seemed to be not only due to its effect on gastric acid secretion but also its effect on mucosal defensive factors like enhanced mucin secretion and decreased cell shedding. As determined by radioimmunoassay, DW did not produce any change in the accumulation of PGE and PGI_2, while HS increased them significantly in the human gastric mucosal incubates compared to NS. However, in the incubates of human colonic mucosa, both DW and HS showed a significant increase in PGE with a tendency to increase in PGI_2 accumulation.

Key words : gastric ulcer, mucin, cell shedding, prostaglandins

INTRODUCTION

The stomach has built-in elements which are destructive to gastric mucus; the acid and pepsin components of the secretion, affect the integrity of the mucus layer (1). There is now evidence that exposure of mild irritants lead to a reduction in the macroscopic signs of mucosal injury caused by subsequent exposure to the necrotizing agents (2, 3, 4). Macroscopically this process described as adaptive cytoprotection, resembles the protection conferred by prostaglandin pretreatment and it has been proposed that adaptive cytoprotection is due to stimulation of endogenous prostaglandin synthesies (5, 6). However, several reports have questioned the involvement of PGs (4, 7, 8) and other endogenous protective products have also been proposed to be involed in adaptive cytoprotection (9, 10). More recently it has been shown that there is a pronounced elevation in gastric mucosal cAMP/cGMP ratio, which is considerd to be an indictor of cytoprotection (11). Hence, the process of adaptation and the integrity of the gastric mucosa and its defence against the injury by irritants still remains controversial. Cytoprotection is the property of therapeutic

*Corresponding Author

agents to protect the gastric mucosa from damaging agents without influencing acid secrection or neutralizing intragastric acidity.

In the present study, we thus investigated the effect of hypotonic (Distilled water, DW) and hypertonic (5% NaCl, HS) solutions on various mucosal defensive factors like cell shedding and mucin secretion in rat gastric ulcer models, besides supporting the evidence of role of PGE and PGI_2 prostanoids by human gastric and colonic mucosa *in vitro*.

METHODS

Animals: Inbred Charles-Foster albino rats (120-160 g), of either sex, were obtained from the central animal house of Institute of Medical Scinences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house (26 ± 2°C, relative humidity 44-56%, light and dark cycles of 10 and 14 h respectively) for 1 week before and during the experiments. Food was withdrawn 18 h before experiment with water *ad libitum*.

Production of gastric lesion and induction of gastric adaptation: Hypotonic (Distilled water, DW) and Hypertonic saline (HS, 5% soudium chloride) solutions at dose of 1 ml/100 g, were administered orally with the help of an orogastric tube given on the day of the experiment in 18 h fasted rats 1 h before the induction of gastric lesions. The gastric ulcers were induced in rats by following methods viz (i) 100% Ethanol (1 ml/ 200 g, 1 h) (12), (ii) Aspirin (200 mg/kg, 4 h) (13), (iii) Cold restraint stress (6 h) (14), and (iv) Pylorus ligation (4 h) (15), while the control animals received normal saline (NS, 1 ml/100 g). In the case of pylorus ligation-induced ulcers DW, HS or NS were given to the animals 15 min before pylorus ligation to see the effect of these treatments on various parameter of offensive-acid and defensive mucosal barrier. After the experiment, animals were sacrificed with an overdose of ether and ulcer index was calculated following the method as described earlier (16). The results of ulcers were analysed statistically by Wilcoxan Sum Rank test method (17).

Gastric secretion study: The gastric juice was collected 4 h after pyloric ligation and centrifuged for 5 min at 2000 rpm and the volume of the supernatant was expressed as ml/100 g body weight. Total acid output was determind by titrating with 0.01 N NaOH, using phenolphthalein as indicator, and is expressed as μEq/4 h. Mucin activity was estimated in the mucosubstances precipitated by treating the gastric juice with 90% ethanol in a 1:9 ratio. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 N NaOH or 1 ml of 0.1 N H_2SO_4. The former was used for the estimation of protein (18), total hexoses (19), hexosamine (20) and fucose (21), while the latter was used for the estimation of sialic acid (22). The results are expressed in μg/ml. The ratio of total carbohydrate (TC, sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity (23, 24). DNA content was estimated and expressed as μg/ml gastric juice/100 g weight of rat (25). Statiststical analysis of data was done by using unpaired Student's 't' test method.

Prostanoid study: Gastric and colonic tissues were taken from surgical specimens removed from patients of benign and malignant disease, at least 5 cm from any macroscopically detected lesion. Patients had not taken any drugs affecting eicosanoid synthesis atleast one week prior to surgery. It was transported to the laboratory in 154 mM NaCl at ambient temperature within 30 min of removal and on arrival, it was transferred to ice-cold phosphate buffer saline pH 7.4 (PBS). The mucosa/submucosa was carefully removed from the underlying muscle while the tissue was bathed in PBS. The mucosa and submucosa was cut into pieces of about 4 mm^2 and washed with PBS which was then drained off. Pieces of 100 mg of mucosa were carefully weighed and pre-incubated in PBS (1 ml, 4°C, for 30 min) which was then drained off, and the tissues were transferred to tubes containing 1 ml of either NS or DW or HS. After further incubation at 37°C for 30 min, the fluid which contained the released eicosanoids was removed and stored at –20°C until assayed (26, 27). Radioimmunoassay method was based on that of Jaffe and Behrman (28), using suitable dilution's of antisera and titrated standards. Assay sensitivities were 10 pg, and the intra-inter-assays coefficients of variation were 6-9% and 10-12%, depending on the eicosanoid measured. All assays were in duplicate. Tritiated prostanoids were purchased from Radiochemical Centre (Amersham, UK). PGE antiserum was obtained fron ICN (High Wycombe, UK) and PGI_2 anti, serum from Wellcome (Beckenham) UK. Since PGE antiserum does not distinguish between PGE_1 and PGE_2 the measurements are expressed as PGE. Results are expressed as mean ± SEM, analysed by Student's 't' test for paired data (2-tailed).

RESULTS

Hypotonic medium (DW) has showed a tendency to decrease the ulcer index in aspirin-, cold restraint stress- and ethanol-

TABLE I :Effect of hypotonic (Distilled water, DW) and hypertonic (5% NaCl, HS) medium on aspirin (ASP, 200 mg/kg, po, 4 h)-, 6 h cold restaint stress (CRS)-, ethanol (ETH, 100%, 1 ml/200 g, po, 1 h)- and 4 h pylorus ligated (PL)- induced gastric ulcers in rats.

	Control (NS)	*DW*	*HS*
		ASP-INDUCED ULCERS (n=10)	
Ulcer index	16.2±4.8	9.9±4.4	5.0±2.0[a]
% inhibition	–	38.9%	69.1%
		CRS-INDUCED ULCERS (n=7)	
Ulcer index	50.2±10.3	31.4±5.0	20.4±6.4[a]
% inhibition	–	37.5%	59.4%
		ETH-INDUCED ULCERS (n=10)	
Ulcer index (mm2/rat)	31.8±5.2	24.6±4.9	6.0±1.9[c]
% inhibition	–	22.6%	81.1%
		PL-INDUCED ULCERS (n=8)	
Ulcer index	10.0±2.3	15.5±3.7	4.5±0.8[a]
% inhibition/ (–) stimulation	–	–55.0%	55.0%

Values are mean ± SEM
Significance : a=P<0.05; c=P,0.001 as compared to their control value.

induced gastric ulcerations in rats, while in case of pylorus ligation-induced gastric ulcers, DW tended to increase ulcer index. On the other hand, HS decreased ulcer index significantly in all the gastric ulcer models studied (Table I).

In human gastric mucosal incubates, DW had little or no effect both on PGE and PGI_2 accumulation, while HS increased them. However, in case of human colonic mucosal incubates, both DW and HS significantly increased the accumulation of PGE but increase in the level of PGI_2 was not significant (Table II).

In pylorus-ligated animals, DW treatment tended to decrease the acid output, DNA and protein content, but showed little or no change in individual or total carbohydrates

TABLE II: Effect of DW and HS on the accumulation of human gastric and colonic mucosal prostaglandins E (PGE) and I_2 (PGI_2).

Treatment	*n*	*Gastric mucosa (ng/g wet tissue, 30 min)*		*Colonic mucosa (ng/g wet tissue, 30 min)*	
		PGE	*PGI_2*	*PGE*	*PGI_2*
Control (NS)	6	102.8±10.9	114.1±21.9	56.0±10.1	90.0±32.8
DW	6	113.1±9.0	118.0±113.1	100.1±17.7[a]	167.0±20.1
HS	6	140.2±8.0[c]	149.0±5.0[c]	110.1±15.0[c]	169.1±16.1

Values are mean ± SEM

Significance : a=P<0.05; c=P<0.001 as compared to their control value.

TABLE III: Effect of DW and HS on various gastric secretion parameters in 4 h pylorus-ligated rats.

Gastric juice	*Control (NS)*	*DW*	*HS*
Volume (ml/100 g body wt)	2.32±0.27	1.81±0.11	2.73±0.32
Acid output (µEq/4h)	221±38	166±15	61±5[c]
DNA (µg/ml/100 g body wt)	243±33	164±29	89±23[b]
Mucin activity (µg/ml)			
Total hexoses	397±38	377±40	492±66
Hexosamine	170±15	165±17	220±36
Fucose	74.2±7.6	60.0±3.8	83.4±12.8
Sialic acid	35.8±2.1	38.3±2.6	50.1±3.0[b]
Total carbohydrate (TC)	677±27	640±99	845±90
Protein (P)	529±56	433±83	382±26[a]
TC : P	1.38±0.24	1.48±0.20	2.24±0.28[a]

Values are mean ± SEM

Significance: a=P<0.05;n b=P<0.01; c=P<0.001 as compared to their control value n=8 each.

or in total carbohydrate : protein ratio. However, HS treatment caused a significant decrease in acid output, DNA and protein content and tended to increase total hexoses, fucose and hexosamine, while sialic acid and total corbohydrate : protein ratio was increased significantly (Table III).

DISCUSSION

Restraint stress to animals has been implicated to enhance acid secretion and decrease mucosal blood flow (29). Ethanol produces damage to gastrointestinal mucosa by various contributing factors which includes mucosal blood flow, platelet thromboxane, damage to capillary endothelium and release of LTC_4/D_4 and platelet activating factor (1). Aspirin has been reported to cause mucosal damage by affecting PGs synthesis, enhanced acid secretion, increase back diffusion of H^+ ions, decrease mucin secretion and breaking of mucosal barrier etc. While pylorus ligation has been reported to affect mucsal blood flow and enhanced acid secretion (1, 30).

Pretreatment with mild irritants has shown to protect the rat gastric mucosa to various necrotizing agents and this protection was shown to be due to the increased generation of PGs which are involved in adaptive cytoprotection (10, 11). Thus, there has been a discrepancy in the role of various mucosal defensive factors and it is possible that not only PGs but other defensive factors like enhanced mucosal blood flow, mucous secretion, mucosal restitution or proliferation, or decrease mucosal exfoliation and acid secretion might be playing role in adaptive cytoprotection.

Our results on PGs are, thus, in confirmity with that of earlier reported studies, where mild irritants were shown to protect the gastric mucosa to various noxious agents (3). The ulcer protection afforded by HS and not by DW in the various gastric ulcer models studied, together with their effects on PGE and PGI_2 (increased by HS but not by DW) does corroborate the role played by these prostanoids in ulcer protection. The differential effect on PGE by DW on gastric mucosa compared to the colonic mucosa may reflect a smaller osmotic perturbation of the gastric mucosal cell membranes. It seems that the gastric mucosa is designed to withstand the osmotic effect of the intraluminal water, whereas the colonic mucosa is normally exposed only to iso-osmotic solutions.

The status of mucin secretion was studied by estimating different fractions of mucosubstances viz. the total hexoses, hexosamine, fucose, sialic acid and protein in the alcoholic precipitate of gastric juice, the ratio between the total carbohydrates (sum of total hexoses, hexosamine, fucose, sialic acid) to protein reflects the functional integrity of the mucosal barrier and serve as a reliable index of mucosal resistance (25) and thus, an incerase in TC : P ratio by HS reflects an increase in mucin secertion. DNA content of the gastric juice is one of important marker of gastric mucosal damage or cell shedding, which is

augmented by ulcerogenic agents and reduced by ulcer protective agents (1, 25). The decrease in DNA content of gastric juice by 5% sodium chloride indicates the cytoprotective role of HS. Thus, the cytoprotection afforded by HS was not only due to its effects on mucosal offensive acid secretion but also on defensive mucin secretion and cell shedding besides its effect on PGs.

ACKNOWLEDGMENTS

RKG is thankful to INSA, New Delhi and the Royal Society, London for Scientist Exchange Fellowship. The authors thank Dr. I. A. Tavares, Senior Lecturer, Academic Department of Surgery, King's College School of Medicine & Dentistry, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NU, for laboratory facilities to complete the work on RIA.

REFERENCES

1. Goel RK, Bhattacharya SK. Gastroduodenal mucosal defence and mucosal protective agents. *Indian J Exp Biol* 1991; 29 : 701–714.
2. St John DJB, Yeomans ND, Mc Dermott FT, De Boer WGRM. Adaptation of the gastric mucosa to repeated administration of aspirin in the rat. *Dig Dis* 1973; 18 : 881–885.
3. Robert A, Nezamis JE Lancaster C, Davies JP, Field SO, Hanchar AJ. Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated by prostaglandins. *Am J Physiol* 1983; 245 : G113–G121.
4. Robert A. Cytoprotection and adapted cytoprotection.In:MelisGF, Boeve J, Misiewicz JJ, (eds) Peptic ulcer disease: basic and clinical aspects.Dordrecht, Boston, Lancaster, U.K: Martinus Nijhoff 1985; 290–316.
5. Konturek SJ, Brozozwski T, Piaztucki I, Radecki T, Dembinski A, Dembinska Kiec A. Role of locally generated prostaglandins in adaptive gastric cytoprotection. *Dig Dis Sci* 1982; 27 : 967–971.
6. Miller TA, Henagan JM. Indomethacin decreases resistance of gastric barrier to disruption by alcohol. *Dig Dis Sci* 1984; 29 : 141–149.
7. Rees WDW, Gibbons LC, Warhurst G, Turnberg LA. Studies of bicarbonate secretion by the normal human stomach *in vivo*. Effect of aspirin, sodium taurocholate and prostaglandin E2 In : Allen A, Flemstrom G, Garnar A, Silen W, Turnberg LA (eds) Mechanism of gastric mucosal protection in the upper gastricintestinal tract. London: Raven, 1984; 119–124.
8. Hawkey CJ, Kemp RT, Walt RP, Bhaskar NK, Davies J, Filipowicz B. Evidence that adaptive cytoprotection in rat is not mediated by prostaglandins. *Gastroenterol* 1988; 94: 948–954.
9. Wallace JL. Increased resistance of the rat gastric mucosa to hemorrhagic damage after exposure to an irritant. Role of the "mucoid cap" and prostaglandin synthesis. *Gastroenterol* 1988; 94 : 22–32.
10. Balaa MA. Adaptive cytoprotection in the rat stomach causes the release of cytoprotective factors in the gastric lumen (abstr). *Gastroenterol* 1988; 94 : A18.
11. Balint GA. Gastric anti-ulcerogenic drug effect. A possible mechanism of its molecular basis. *Acta Physiologica Hungarica* 1992; 80 : 81–88.
12. Hollander D, Tarnawski A, Krause WJ, Gergely H. Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. *Gastroenterol* 1985; 88 : 366–374.
13. Goel RK, Govinda Das D, Sanyal AK. Effect of vegetable banana powder on changes induced by ulcerogenic agents on dissolved mucosubstancees in gastric juice. *Indian J Gastroenterrol* 1985; 4 : 249–251.
14. Amar A, Sanyal AK. Immobilisation stress in rats: Effects on rectal temperature and possible role of brain monoamines in hypothermia. *Psychopharmacology* 1981; 73 : 157–160.
15. Sanyal AK, Debnath PK, Bhattacharya SK, Gode KD. The effect of cyproheptadine on gastric activity. An experimental study, In: C.J. Pfeiffer (Ed.), Peptic ulcer, edited by CJ Pfeiffer, Munksgaard, Copenhagen 1971; 312–318.
16. Sanyal AK, Pandey BL, Goel RK. The effect of a traditional preparation of copper, tamra-

bhasma, on experimental ulcers and gastric secretion. *J Ethnopharmacol* 1982; 5: 79–89.

17. Padmanabha Pillai N, Ramaswamy S, Gopalakrishnan V, Ghosh MN. Effect of cholinergic drugs on acute and chronic morphine dependence. *Arch int Pharmacodyn* 1982; 257 : 146–154.
18. Lowery OH, Rosenborugh NI, Farr AL, Randal RJ. Protein measurement with folin phenol reagent. *J Biol Chem* 1951; 193 : 265–275.
19. Winzler RJ. Determination of serum glycoproteins. *Methods Biochem Anal* 1958; 2 : 279–311.
20. Dische Z, Borenfreund E. A spectroscopic method for the microdetermination of hexosamine. *J Biol Chem* 1950; 184 : 517–522.
21. Dische Z, Shettles LB. A specific colour reaction for methyl pentoses and spectrophotometric micro method for their determinations. *J Biol Chem* 1948; 175: 595–597.
22. Warren L. The thiobarbituric acid assay of sialic acids. *J Biol Chem* 1959; 234 : 1971–1975.
23. Sanyal AK, Mitra PK, Goel RK. A modified method to estimate dissolved mucosubstances in gastric juice. *Indian J Exp Biol* 1983; 21 : 78–80.
24. Goel RK, Chakrabarthy A, Sanyal AK. The effect of biological variables on the antiulcerogenic effect of vegetable plantain banana. *Planta Medica* 1985; 2 : 85–88.
25. Mukhopadhyay K, Bhattacharya D, Chaktabarti A, Goel RK, Sanyal AK. Effect of banana powder (*Musa sapientum var.* paradisiaca) on gastric mucosal shedding. *J Ethnopharmacol* 1987; 21 : 11–19.
26. Tavares IA, Collins PO, Bennett A. Inhibition of prostanoid synthesis by human gastric mucosa. *Aliment Pharmacol Ther* 1987; 1 : 617–625.
27. Goel RK, Tavares IA, Bennett A. Effect of ethanol on eicosanoid synthesis by human gastric and colonic mucosal pieces. *Br J Pharmacol* 1990; 99 : 289–292.
28. Jaffe BM, Behrman MR. Methods of hormone radioimmunoassay (Academic Press, New York)1974; 19–34.
29. Guth P. In Peptic ulcer (ed.), CJ Pfeiffer, Munnksgaard, Copenhagen 1971; 221–236.
30. Boughton Smith NK, Whittle BJR. Inhibition by 16, 16-dimethyl PGE_2 of ethanol-induced gastric mucosal damage and leukotriene B_4 and C_4 formation. *Prostaglandins* 1988; 35 : 945–957.