

## PROTECTIVE EFFECT OF PROPRANOLOL ON ETHANOL-INDUCED GASTRIC LESIONS IN RATS : PROBABLE MECHANISM OF ACTION

PADMANABH V. RATABOLI, PADMA N. BHANDARE,  
ROSA S. DINIZ D'SOUZA\* AND VISHWANATH G. DHUME

Department of Pharmacology,  
Goa Medical College, P.O. Bambolim Complex,  
Tiswadi, Goa - 403 202

( Received on June 1, 1991 )

**Abstract :** The non-selective  $\beta$ -adrenoceptor antagonist, propranolol, has been reported to protect against gastric injury in mice, an effect only partly due to prostaglandin release. This study was designed to confirm the gastric cytoprotective effect of propranolol in another species of animal, the rat, and investigate further its mechanism of action. Our results show that propranolol prevents both ethanol-induced gastric lesions as well as ethanol-induced contraction of the circular muscle of rat fundic strip. The local anaesthetic, lignocaine also inhibited the effect of ethanol on circular muscle. However, timolol, another non-selective  $\beta$ -adrenoceptor antagonist, failed to produce such an action. The effect of propranolol was abolished by the cyclooxygenase inhibitor, indomethacin and a high dose of the guanylate cyclase inhibitor, methylene blue. The results suggest that in addition to prostaglandins, endogenous nitric oxide and the membrane stabilising action of propranolol may also be involved in its gastroprotective action.

**Key words :** propranolol      gastric lesion      ethanol      circular muscle

### INTRODUCTION

We have previously reported that the non-selective  $\beta$ -adrenoceptor antagonist, propranolol, exerts a protective effect against ethanol-induced gastric lesions in mice (1). This effect is not due to  $\beta$ -adrenergic blockade and is only partly prevented by the cyclooxygenase inhibitor, indomethacin, showing that in addition to prostaglandins, other mechanism may be involved.

Gastric injury caused by necrotising agents consistently occurs as band-like lesions at the crest of mucosal folds and is preceded by gastric hypercontractions (2,3). Various studies have shown that decreased gastric motility leading to a flattening of mucosal folds is associated with gastric cytoprotection (3, 4, 5).

In the course of our experiments with propranolol in mice, we observed a flattening of mucosal folds which posed a question : Is the gastric cytoprotective action of propranolol due to a decrease in

gastric motility? This made us to take up this study which was designed I) To confirm protective effect of propranolol against ethanol-induced lesions in another species of animal namely, the rats, II) As mucosal folds relate closely to circular muscle actions (2), to find out the effect of propranolol on ethanol-induced contraction of circular muscle of rat fundic strip if any, and use this parameter to further investigate the mechanism of the gastro-protective effect of propranolol.

### METHODS

Male Wistar rats (200-250 g) were randomly divided into groups of six. They were deprived of food for 24 hr prior to drug administration but were allowed free access to water.

*Induction of gastric lesions :* Animals were administered 1 ml of 100% ethanol orally and killed after one hr. The stomachs were then opened along the greater curvature, rinsed with normal saline and pinned on a flat surface. The length and width of each red

\*Corresponding Author

haemorrhagic band was then transposed on a cardboard with the help of callipers and measured with a scale to find out its area. The total area of haemorrhagic bands was then summed up for each stomach. While one of us performed the experiments, the other two assessed each coded stomach separately.

Control animals received normal saline (1 ml/kg) and test animals propranolol (2.5, 5.0 and 10.0 mg/kg, ip) 30 min before the administration of ethanol.

**Contraction of circular muscle strip of rat fundus:** The fundic strip was prepared by modifying the method of Vane (6) in that instead of longitudinal muscle, the strip consisted of circular muscle. The fundic strip was then mounted in a 10 ml bath containing Tyrode solution at 37°C, bubbled with air and allowed to equilibrate for one hr before the below mentioned experiments were carried out with a cycle of 60 sec recording and 10 min relaxation.

Contraction of circular muscle was produced by addition of 100% ethanol to the bath in doses ranging from 0.02 to 0.14 ml/ml of bath. Ethanol-induced contractions were dose-dependent and reproducible. There was no tachyphylaxis. Maximal contraction was produced by 0.14 ml. Concentrations beyond this damaged the tissue and responses became smaller. Hence in subsequent experiments doses upto 0.1 ml were used. Dose response curves were obtained with ethanol alone and in the presence of propranolol (2.5, 5.0 and 10 µg/ml of bath, timolol (5.0 and 10 µg/ml) and lignocaine (10 and 20 µg/ml). To investigate further the mechanism of action of propranolol, the tissue was incubated either with the inhibitor of soluble guanylate cyclase, methylene blue (5 and 10 µg/ml of bath, for 15 min) or the cyclooxygenase inhibitor, indomethacin (1 µg/ml of bath for 20 min) and experiments with propranolol repeated.

**Statistical analysis:** All the data are expressed as mean ± S.E.M. and analysed using Student's t-test.

## RESULTS

Table I shows the effect of three doses of propranolol on ethanol-induced gastric haemorrhagic lesions in the rat. It can be seen that propranolol had a significant protective effect in all the three doses used as compared to controls. However, there was

no significant difference between the effect of 2.5 and 5 mg/kg.

TABLE I: Effect of propranolol on ethanol-induced gastric haemorrhagic lesions.

Drug	Dose	Area of haemorrhagic lesions (mm) Mean ± S.E.
Saline	1 ml/kg	73 ± 2.9
Propranolol	2.5 mg/kg, ip	34.5 ± 2.5*
	5 mg/kg, ip	29.6 ± 3.2*
	10 mg/kg, ip	14.2 ± 3.8*

n = 6, P < 0.001 as compared to control.

The inhibitory effect of propranolol and lignocaine on ethanol-induced contraction of circular muscle of rat fundus can be seen in Fig. 1. Propranolol produced a significant, dose-dependent shift to the right in the dose-response curve to ethanol with inhibition of maxime. Recovery of the tissue from the effect of propranolol was delayed (30 min of repeated washing). Timolol had no effect of ethanol-induced contractions in the doses used (not shown in the figure). Lignocaine had a similar effect to that of propranolol, however it was less potent and the effect was rapidly reversible on washing.

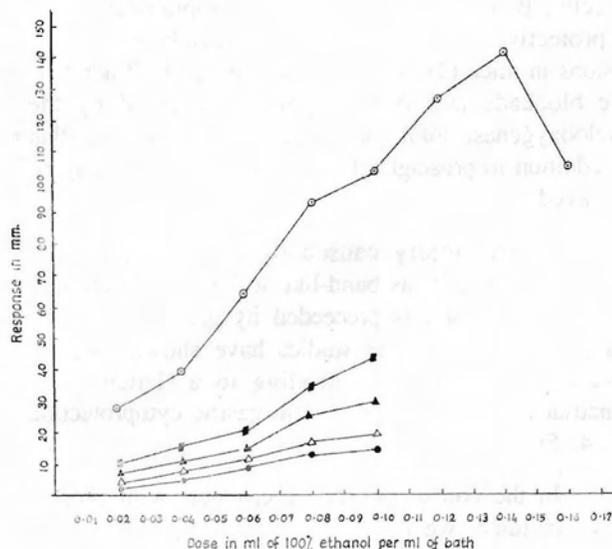


Fig 1: Effect of 100% ethanol on circular muscle of rat fundus perse (○) and in presence of propranolol 2.5 (■), 5 (▲) and 10 µg/ml of bath (●) and lignocaine 20 µg/ml of bath (△).

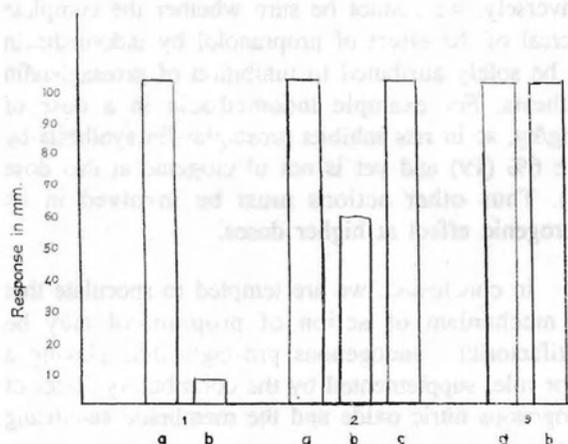


Fig 2: Effect of 100% ethanol (0.1 ml/ml of bath) on circular muscle of rat fundus : 1a) *per se*, 1b) in presence of propranolol 10 µg/ml of bath.

2 a, b – after incubation with methylene blue 5 µg/ml of bath a) *per se*, b) in presence of propranolol 10 µg/ml of bath.

2c) after incubation with 10 µg/ml of bath methylene blue and in presence of propranolol 10 µg/ml of bath.

3 a,b – after incubation with indomethacin 1 µg/ml of bath : a) *per se*, b) in presence of propranolol 10 µg/ml of bath.

Fig. 2. shows the modifications by methylene blue and indomethacin of the effect of propranolol on ethanol-induced contractions. Both methylene blue and indomethacin did not affect ethanol-induced contraction *per se*. However, methylene blue (5 µg/ml) partially prevented the inhibitory effect of propranolol. A high dose of methylene blue (10 µg/ml) and indomethacin (1 µg/ml) abolished the effect of propranolol.

## DISCUSSION

There are conflicting reports on the effect of propranolol on ethanol-induced gastric lesions. Pre-treatment with propranolol blocked the protective effect of topical isoproterenol on ethanol-induced gastric injury in rats (7). On the other hand, some studies have shown that propranolol reduced ethanol-induced gastric damage in portal hypertensive rats but had no effect in sham-operated controls (8,9).

Our present study in rats confirms the protective effect of propranolol previously reported by us in mice (1) rulling out species specificity.

Various studies have suggested that changes in gastric motility may play a role in the development and prevention of experimental gastric lesions (2,4,10). Relaxation of circular muscle may protect the gastric mucosa through flattening of the folds. This will increase the mucosal surface area exposed to necrotising agents and reduce the volume of the irritant on the rugal crests. Such an action has been postulated to play a role in the cytoprotective effect of prostaglandins (3). Our study shows that ethanol produces a marked contraction of the circular muscle of rat fundic strip. Such a contraction can lead to "mucosal compression" at the site of the greatest mechanical stress, that is at the crests of mucosal folds leading to necrosis and ulceration (2). In this study the non-selective β-adrenergic antagonist propranolol dose-dependently inhibited the contractile effect of ethanol on circular muscle strip of rat fundus. However, another non-selective β-adrenergic antagonist, timolol failed to produce such an effect suggesting that β-receptors are not involved. Unlike timolol, propranolol is known to exert a membrane-stabilising action at higher doses. To ascertain the role of such an action of propranolol, we studied the effect of the local anesthetic lignocaine. Like propranolol, lignocaine also produced a dose-dependent inhibition of ethanol-induced contractions. However, it was less potent and its effect was rapidly reversible on washing. Thus a local anesthetic activity cannot entirely account for the action of propranolol.

Endogenous prostaglandins have been proposed as one of the mediators of cytoprotection (11). Recently, studies have focused on another possible endogenous modulator of gastric mucosal integrity, nitric oxide (12) which has been shown to activate soluble guanylate cyclase and play a vasodilator role in gastric microcirculation (13). To investigate the possible involvement of these two endogenous mediators in the gastroprotective action of propranolol, we studied the effect of the cyclo-oxygenase inhibitor indomethacin and the guanylate cyclase inhibitor methylene blue. Indomethacin (1 µg/ml) completely

prevented the action of propranolol on ethanol-induced circular muscle contraction, suggesting the involvement of the prostaglandin pathway. This is in agreement with other studies which have shown a relationship between propranolol and prostaglandins (14, 15, 16). The effect of propranolol was only partially prevented by a conventional dose (5 µg/ml) of methylene blue (17). However a higher dose (10 µg/ml) completely abolished the effect. Though methylene blue is widely used as a pharmacological tool to inhibit soluble guanylate cyclase and thus interfere with responses to nitric oxide, the drug has also been reported to exert other actions such as inhibition of PGI<sub>2</sub> synthesis in dog renal artery strip (18). Thus the need to use a higher dose of methylene blue to abolish the effect of propranolol probably indicates an

additional effect on prostaglandin synthesis. Conversely, we cannot be sure whether the complete reversal of the effect of propranolol by indomethacin can be solely attributed to inhibition of prostaglandin synthesis. For example indomethacin in a dose of 5 mg/kg, sc in rats inhibits prostaglandin synthesis by 88 ± 6% (19) and yet is not ulcerogenic at this dose (20). Thus other actions must be involved in its ulcerogenic effect at higher doses.

In conclusion, we are tempted to speculate that the mechanism of action of propranolol may be multifactorial : endogenous prostaglandins playing a major role, supplemented by the contributory effect of endogenous nitric oxide and the membrane stabilising action.

#### REFERENCES

- Bhandare P, Diniz D'Souza R, Mainker A, Dhume V. Protective effect of propranolol on ethanol-induced gastric lesions in mice. *Eur J Pharmacol* 1990; 191 : 167-172.
- Mersereau WA, Hinchey EJ. Role of gastric mucosal folds in formation of focal ulcers in the rat. *Surgery* 1982; 91: 150-155.
- Takeuchi K, Nobuhara Y. Inhibition of gastric motor activity by 16, 16-dimethyl prostaglandin E<sub>2</sub> : a probable explanation of cytoprotection. *Dig Dis Sci* 1985; 30: 1181-1188.
- Takeuchi K, Ueki S, Okabe S. Importance of gastric motility in the pathogenesis of indomethacin-induced gastric lesions in rats. *Dig Dis Sci* 1986; 32 : 1114-1122.
- Takeuchi K, Nishiwaki K, Okabe S. Effect of dopamine on gastric mucosal lesions induced by ethanol in rats-possible involvement of antigastric motor activity mediated with alpha<sub>2</sub> adrenoceptors. *Dig Dis Sci* 1987; 31: 1100-1107.
- Vane JR. A sensitive method for the assay of 5-hydroxytryptamine. *Br J Pharm* 1957; 12 : 344-349.
- Howard JT, Passaro E, Guth PH. Topical isoproterenol protects the rat gastric mucosa from ethanol-induced injury. *J Surg Res* 1989; 46 : 640-643.
- Sankary H, Sarfeh IJ, Tamawaski A, Maeda KJ, Ivey KJ, Mason GR. Propranolol reduces ethanol-induced gastric mucosal damage in portal hypertensive rats. *Dig Dis Sci* 1986; 31 (2) : 162-165.
- Geoffrey P, Duchateau A, Thieffin G, Zeitoun P. Effects of propranolol and sucralfate on ethanol-induced gastric mucosal damage in chronic portal hypertensive rats. *J Hepatol* 1987; 2: 162-166.
- Garrick T, Buack S, Bass P. Gastric motility is a major factor in cold-restraint induced lesion formation in rats. *Am J Physiol* 1986; 250 : 6191-6199.
- Robert A. Cytoprotection by prostaglandins. *Gastroenterology* 1979; 77: 761-767.
- Whittle BJR, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide : interactions with prostanoids and sensory neuropeptides in the rat. *Br J Pharmacol* 1990; 99 : 607-611.
- Pique JM, Whittle BJR, Espluges JV. The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. *Eur J Pharmacol* 1989; 74 : 293-296.
- Riesterer L, Jaques R. Interference by β-adrenergic blocking agents with the anti-inflammatory action of various drugs. *Helv Physiol Pharmacol Acta* 1968; 26 : 287-292.
- Webster J. Interaction of NSAIDs with diuretics and β-blockers : mechanism and clinical implications. *Drugs* 1985; 30: 32-36.
- Jackson EK, Campbell WB. A possible antihypertensive mechanism of propranolol : antagonism of Angiotensin II, enhancement of sympathetic nerve transmission through prostaglandins. *Hypertension (Dallas)* 1981; 31 : 23-26.
- Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ. Relationship between cyclic guanosine 3'5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide : effects of methylene blue and methemoglobin. *J Pharmacol exp Ther* 1981; 219 : 181-186.
- Okamura J, Yoshida K, Joda N. Suppression by methylene blue of prostaglandin I<sub>2</sub> synthesis in isolated dog renal arteries. *J Pharmacol Exp Ther* 1990; 254: 198-203.
- Morris GP, Keenan CM, Wallace KM, Wallace JL, Williamson TE. Protection of rat gastric mucosa by sucralfate : Effects of luminal stasis and of inhibition of prostaglandin synthesis. *Am J Med* 1989; 86 (Suppl 6A) : 10-13.
- Nemeroff CB, Hernandez DE, Orlando RC, Prange AR. Cytoprotective effect of centrally administered neurotensin on stress-induced gastric ulcers. *Am J Physiol* 1982; 242 : G 342-345.