Bromophenacyl bromide, a phospholipase A2 inhibitor attenuates chemically induced gastroduodenal ulcers in rats

Mohammad Tariq, Ibrahim Elfaki, Haseeb Ahmad Khan, Mohammad Arshaduddin, Samia Sobki, Meshal Al Moutaery

AIM: To study the effect of bromophenacyl bromide (BPB), a phospholipase A2 inhibitor on gastric secretion and to protect chemically induced gastric and duodenal ulcers in rats.

METHODS: Acid secretion studies were undertaken in pylorus-ligated rats with BPB treatment (0, 5, 15 and 45 mg/kg). Gastric and duodenal lesions in the rats were induced by ethanol and cysteamine respectively. The levels of gastric wall mucus, nonprotein sulfhydryls (NP-SH) and myeloperoxidase (MPO) were also measured in the glandular stomach of rats following ethanol induced gastric lesions.

RESULTS: BPB produced a dose-dependent inhibition of gastric acid secretion and acidity in rats. Pretreatment with BPB significantly attenuated the formation of ethanol induced gastric lesion. BPB also protected intestinal mucosa against cysteamine-induced duodenal ulcers. The antiulcer activity of BPB was associated with significant inhibition of ethanol-induced depletion of gastric wall mucus, NP-SH and MPO. These findings pointed towards the mediation of sulfhydryls in BPB induced gastrointestinal cytoprotection.

CONCLUSION: BPB possesses significant antiulcer and cytoprotective activity against experimentally induced gastroduodenal lesions.

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Key words: Bromophenacyl bromide; Phospholipase A2; Gastric secretion; Gastric ulcer; Duodenal ulcer; Sulfhydryls; Myeloperoxidase


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INTRODUCTION

Phospholipids play an important role in the preservation of gastrointestinal homeostasis[1]. The gastric mucosa has a hydrophobic lining which is assumed to have protective functions against luminal acid as well as intrinsic and extrinsic corrosive agents[2,3]. The hydrophobicity of the mucosal lining is attributed to a surfactant like phospholipid monolayer adsorbed to the mucosal surface which acts as a mucosal barrier and impedes back-diffusion of luminal H+ into the mucosal tissue and defends gastric mucosa against damage induced by strong acids[4] and other barrier breaking agents[5-8]. Altered phospholipids profile of gastric mucosa has been noticed in clinical gastropathies including H pylori induced gastritis and peptic ulcers[9]. Injury of the intestinal surface layer leads to an inflammatory reaction that is characterized by a variety of inflammatory mediators, activation of complement cascade system and of lipid mediator synthesis[10]. In addition, damage of the gastrointestinal surface protection system and the breakdown of complex membrane lipids activate phospholipase A2 (PLA2), a key enzyme in the production of inflammatory lipid mediators[11]. High concentrations of PLA2 have been reported in gastric mucosa[12]. PLA2 mediated hydrolysis of membrane lipids results in membrane perturbation, cell degranulation and stripping of cell surface receptors[13]. Intracellular PLA2 plays an important role in inflammation by acting upon cell membrane to release arachidonic acid from membrane phospholipids for the synthesis of eicosanoids, lipophospholipids and platelet activating factor[14,15]. Arachidonic acid serves as a primary substrate for eicosanoid production yielding prostaglandins, leukotrienes and lipoxins; all of them have multiple vasoactive and cellular regulatory functions[16-18]. There is abundant evidence suggesting the role of mucosal microcirculatory disturbances in experimental gastrointestinal damage[19-21].
Exposure of gastric mucosa to necrotizing agents results in edema, vacuolization and necrosis of the luminal epithelial cells; these lesions morphologically resemble those caused by ischemic-reperfusion injury in the intestine. The ischemia is known to deplete tissue adenosine triphosphate (ATP) and significantly increase its metabolic product hypoxanthine, which in the presence of xanthine oxidase results in the production of xantine and highly reactive superoxide radicals. Ischemic insult to intestinal mucosa causes excessive production of oxygen derived free radicals (ODFR) that may initiate a chain of reactions in membrane-bound lipids leading to lipid peroxidation and PLA₂ activation. Inhibition of this cascade by scavenging ODFR and/or inhibiting PLA₂ could be an effective tool to protect gastrointestinal mucosa against chemically induced lesions. Earlier studies have shown protective effects of quinacrine, a PLA₂ inhibitor, against ischemia and injury of various organs including heart, stomach, intestine and lungs. Bromophenacyl bromide (BPB) is a PLA₂ inhibitor which is structurally different from quinacrine and inhibits PLA₂ by covalently binding to its active site rather than affecting the enzyme-substrate interface (mode of quinacrine-induced PLA₂ inhibition). The present investigation was aimed to study the effect of BPB on chemically induced gastroduodenal ulcers in rats.

**MATERIALS AND METHODS**

**Animals and dosing**

Albino Wistar rats of either sex, approximately of same age, weighing 150 to 200 g and fed on standard chow diet were used. They were randomly divided into experimental groups of 6 rats each. The aqueous solutions of ulcerogens and BPB were freshly prepared before administration. BPB at doses of 5, 15 and 45 mg/kg was given intraperitoneally for gastric secretion studies and by gavage for antulcer studies. The rats were sacrificed, and the stomachs removed and opened along the greater curvature. After washing with saline the gastric lesions were quantified by a person unaware of the treatments. The protocol of animal studies was approved by the Research and Ethics Committee of Armed Forces Hospital, Riyadh, Saudi Arabia.

**Pylorus ligated rats**

The rats were fasted for 36 h with access to water ad libitum before the pylorus was ligated under ether anesthesia, care being taken not to cause bleeding or to occlude blood vessels. BPB was administered immediately after pylorus ligation (Shay) by ip route. The rats were sacrificed at 6 h after the pylorus ligation. The stomachs were removed; the contents were collected, volumes measured, centrifuged and analyzed for titratable acidity against 0.01 mol/L NaOH to pH 7 and the total acid output was calculated.

**Cysteamine-induced duodenal ulcers**

Duodenal ulcers were induced by two doses of cysteamine hydrochloride (400 mg/kg ig in 10% aqueous solution) at an interval of 4 h according to the method described by Szabo. BPB was administered 30 min before each dose of cysteamine. All the rats were sacrificed 24 h after the first dose of cysteamine and the duodenum was excised carefully and opened along the antimesenteric side. The duodenal ulcers were scored using a scale of 0 to 3, where 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, and 3 = perforated or penetrated ulcer (into the pancreas or liver). The sum of the intensity of each lesion was used as ulcer index.

**Ethanol-induced gastric ulcers**

The rats were administered (ig) with 1 mL of absolute ethanol. BPB was given 30 min before the administration of ethanol. One hour after the administration of ethanol the rats were sacrificed and examined for the lesions in stomachs. The scoring of lesions, assays of gastric wall mucus and sulfhydryls in the stomach were done as follows: The patchel lesions of stomach induced by ethanol were scored according to the method described by Schiantarelli et al using the following scale: 0 = normal mucosa; 1 = hyperemic mucosa or up to 3 small patches; 2 = from 4 to 10 small patches; 3 = more than 10 small or up to 3 medium-sized patches; 4 = from 4 to 6 medium-sized patches; 5 = more than 6 medium-sized or up to 3 large patches; 6 = from 4 to 6 large patches; 7 = from 7 to 10 large patches; 8 = more than 10 large patches or extensive necrotic zones. “Small” was defined as up to 2 mm across (max. diameter), “medium-sized” as between 2 and 4 mm across and “large” as more than 4 mm across.

**Determination of gastric wall mucus**

Gastric wall mucus was determined according to the modified procedure of Corne et al. The glandular segment of the stomach was separated from the lumen of the stomach, weighed, and transferred immediately to 10 mL of 0.1% w/v Alcian blue solution (in 0.16 mmol/L sucrose solution buffered with 0.05 mL sodium acetate at pH 5). Tissue was stained for 2 h in Alcian blue, and excess dye was removed by two successive rinses with 10 mL of 0.25 mmol/L sucrose, first for 15 min and then for 45 min. Dye complexed with the gastric wall mucus was extracted with 10 mL of 0.5 mmol/L magnesium chloride which was intermittently shaken for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 4000 r/min for 10 min and the absorbance of aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated.

**Estimation of nonprotein sulfhydryls**

Gastric mucosal nonprotein sulfhydryls (NP-SH) was measured according to the method of Sedlak and Lindsay. The glandular part of stomach was homogenized in ice-cold 0.02 mmol/L EDTA. Aliquots of 5 mL of the homogenates were mixed in 15 mL test tubes with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid. The tubes were shaken intermittently for 10 min and centrifuged at 3000 g. Two milliliters of supernatant were mixed with 4 mL of 0.4 mol/L Tris buffer at pH 8.9; 0.1 mL of

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DTNB [5, 5'-dithio-bis-(2-nitrobenzoic acid)] was added and the sample was shaken. The absorbance was measured within 5 min of addition of DTNB at 412 nm against a reagent blank.

**Determination of myeloperoxidase activity**

Myeloperoxidase (MPO) activity in the gastric mucosa was measured according to the methods described earlier [31]. Pre-weighed tissue was homogenized (1:10 wt/vol) in 0.5% hexadecyltrimethyl ammonium bromide in 50 mmol potassium phosphate buffer (pH 6.0) before sonication in an ice bath for 20 s. Three freeze/thaw cycles were performed followed by sonication (20 s in ice bath). The samples were centrifuged at 17 000 g (5 min, 4 °C) and MPO in the supernatant was assayed by mixing of 0.1 mL of supernatant with 2.9 mL of 50 mmol/L potassium phosphate buffer (pH 6.0) containing 0.167 g/L o-dianasidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured for 4 min using UV-visible spectrophotometer (UV-160A, Shimadzu, Japan).

**Statistical analysis**

Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. Differences with \( P < 0.05 \) were considered as statistically significant.

**RESULTS**

**Effect of BPB on gastric secretions in 6 h pylorus-ligated rats**

In control rats, pylorus ligation for 6 h resulted in the accumulation of 9.8 ± 0.55 mL of gastric secretions and a total acid output of 663 ± 48.7 mEq (Table 1). The volume of gastric secretion in the rats treated with 5, 15 and 45 mg/kg of BPB significantly reduced to 7.4 ± 0.42 mL, 6.6 ± 0.59 mL and 4.5 ± 0.47 mL respectively (ANOVA, \( F = 17.25, P < 0.001 \)). A significant decrease in total acid output was observed in the rats treated with 15 mg/kg (193 ± 51.5 mEq) and 45 mg/kg (162 ± 34.8 mEq) of BPB (ANOVA, \( F = 26.71, P < 0.001 \)). The rats treated with 5 mg/kg of BPB failed to show any significant change (576 ± 66.9 mEq) in total acid output as compared to control group (Table 1).

**Effect of BPB on cysteamine-induced duodenal ulcers**

Administration of cysteamine produced elongated lesions extending longitudinally down the duodenum. The lesion area of the rats in control group was 33.3 ± 0.21 mm². Treatment of rats with BPB reduced the area of lesions in all the groups. A significant reduction in lesion area was observed in the rats treated with 15 mg/kg (193 ± 51.5 mEq) and 45 mg/kg (162 ± 34.8 mEq) of BPB (ANOVA, \( F = 17.45, P < 0.001 \)). The decrease in lesion area of the rats treated with 5 mg/kg of BPB failed to show any significant change (576 ± 66.9 mEq) in total acid output as compared to control group (Table 1).

**Effect of BPB on ethanol-induced gastric lesions**

The treatment of rats with absolute ethanol produced extensive gastric lesions in the glandular mucosa of stomach in all the control rats. The ulcer index was 8.0 ± 0.31 in control rats 1 h after ethanol administration. Pretreatment of rats with BPB at the doses of 5 mg/kg (ulcer index = 3.0 ± 0.63), 15 mg/kg (0.6 ± 0.24) and 45 mg/kg (0.0 ± 0.0) significantly inhibited the formation of gastric lesions (ANOVA, \( F = 72.07, P < 0.001 \)) (Figure 2). Ethanol-induced lesions were characterized by multiple

<table>
<thead>
<tr>
<th>Table 1 Effect of BPB on gastric secretion and acidity in 6 h pylorus ligated rats (mean ± SE)</th>
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<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Control (Ligation only)</td>
</tr>
<tr>
<td>Ligation + BPB 5 mg/kg</td>
</tr>
<tr>
<td>Ligation + BPB 15 mg/kg</td>
</tr>
<tr>
<td>Ligation + BPB 45 mg/kg</td>
</tr>
</tbody>
</table>

\( ^b \)P < 0.01 vs control (Dunnett’s test).
hemorrhagic red bands (patches) of different size along the axis of the glandular stomach (Figure 3). Histological examination of gastric mucosa showed the appearance of these lesions in the form of gastric pits with detachment of the surface epithelium; epithelial cells appeared to be vacuolated and microvessels elongated (Figure 4). Pretreatment with BPB dose-dependently prevented ethanol-induced mucosal damage.

**Effect of BPB on ethanol-induced changes in gastric wall mucus**
The treatment of rats with ethanol significantly decreased the Alcian blue binding capacity of gastric wall mucus (688 ± 25.2 μg Alcian blue/g of tissue) as compared to control rats (935 ± 60.5 μg/g). Pretreatment of rats with BPB at the doses of 5 mg/kg (847 ± 75.8 μg/g), 15 mg/kg (820 ± 70.6 μg/g) and 45 mg/kg (927 ± 34.2 μg/g) significantly enhanced Alcian blue binding capacity of gastric mucosa (ANOVA F = 3.09, P < 0.05, Table 2).

**Effect of BPB on ethanol-induced depletion of gastric mucosal NP-SH**
The level of NP-SH in the gastric mucosa of control rats was 4.26 ± 0.11 μmol/g of tissue, which was significantly decreased to 2.96 ± 0.22 μmol/g following the administration of ethanol. Pretreatment of rats with BPB at all the three doses significantly inhibited ethanol-induced depletion of NP-SH (ANOVA F = 13.76, P < 0.001, Table 2).

**Effect of BPB on ethanol-induced changes in gastric MPO activity**
Changes in gastric accumulation of leukocytes following ethanol-induced lesions were evaluated by measurement of gastric MPO activity, which was significantly increased as compared to control mucosa (Table 2). Pretreatment with BPB significantly attenuated ethanol-induced increase in gastric MPO activity in rats (ANOVA, F = 8.16, P < 0.001).
activity results in breakdown of membrane phospholipids across the epical membrane is coupled with the move-membranes substantial role of ODFR and PLA2 in mediating ethanol-lesions (Figures 2-4). Numerous studies have indicated BPB protected them against ethanol-induced gastric such, from its interaction with the adhering gastric mucus the cytoprotective activity of this BPB could result, at least in such as ethanol and indomethacin mechanisms termed as the ‘aggressive factor’. Pathogenesis of gastric and duodenal ulcers and is often increase in gastric acidity decrease in the volume and acid output of gastric secre-pretreatment with BPB produced a dose dependent increase in the volume and acid output of gastric secretions in Shay rats (Table 1). The increase in gastric acidity is considered as an important contributing factor in the pathogenesis of gastric and duodenal ulcers and is often termed as the ‘aggressive factor’. The regulation of gastric acid secretion is complex and maintained by endogenous gastrin, histamine, somatostatin and cholinergic mechanisms. For acid secretion the expulsion of H+ across the epical membrane is coupled with the movement of K+ into the cell. Phospholipase A2 inhibitors are known to modulate proton transport across cell membranes and PLA2 has been shown to inhibit PLA2 mediated histamine release from parietal cell in rats. A significant protective effect of BPB was observed against cysteamine-induced duodenal ulcers (Figure 1). The pathogenesis of cysteamine-induced duodenal lesions is far from clear. Cysteamine ulcers are considered to be associated with the hypersecretion of gastrin and hydrochloric acid and decreased mucosal resistance. The antiulcer activity of BPB may to some extent be attributed to its ability to inhibit gastric acid secretions (Table 1) and to preserve mucosal integrity (Table 2). Our results revealed that BPB significantly protected gastric mucosa against the depletion of gastric wall mucus (Table 2). The mucus gel adhering to the gastric mucosal surface protects the underlying epithelium against acid, pepsi and necrotizing agents such as ethanol and indomethacin. It plays a more important role in defense of gastric mucosa against chemical or mechanical aggressions than the soluble mucus found in the lumen of the stomach. The gastric mucus coat is thought to be important in facilitating the repair of the damaged gastric epithelium. It seemed likely that the cytoprotective activity of this BPB could result, at least in part, from its interaction with the adhering gastric mucus layer. Our results revealed that pretreatment of rats with BPB protected them against ethanol-induced gastric lesions (Figures 2-4). Numerous studies have indicated substantial role of ODFR and PLA2 in mediating ethanol-induced intestinal mucosal injury. The enhanced PLA2 activity results in breakdown of membrane phospholipids and activation of arachidonic acid cascade generating leukotrienes, prostaglandins and lipoxins. Prostacyclin is the major vasodilator and inhibitor of platelet aggregation, whereas thromboxan A2 has the opposite effects. The ability of BPB to inhibit this proinflammatory cascade might be responsible for protecting gastric mucosa against chemically-induced lesions. The cytoprotective effect of BPB was accompanied by attenuation of ethanol-induced increase in MPO, a marker of neutrophil activity (Table 2). Neutrophils are the major inflammatory cell type infiltrating the injured mucosa following exposure to ethanol. Strategies to counteract neutrophils infiltration/activation have been shown to protect animals against gastric ulcers. A significant decrease in gastric NP-SH following ethanol administration indicated massive generation of ODFR (Table 2). Our findings are in agreement with earlier reports showing depletion of sulphydryls in ethanol-induced gastric lesions. Within an inflammatory process, ODFRs are generated and initiate a chain reaction within membrane-bound lipids leading to lipid peroxidation. Treatment of rats with glutathione depletors has been shown to significantly potentiate ulcerogen-induced gastric mucosal injury, whereas increase in mucosal NP-SH exerts gastroprotective effect. Sulphydryl compounds play an important role in the formation of gastrointestinal mucus, which protects underlying gastric mucosa against necrotizing agents. Moreover, sulphydryls have the ability to scavenge ODFR produced in tissues following the exposure to cytotoxic compounds. These observations clearly point towards the mediation of sulphydryls in BPB induced gastroprotection.

In conclusion, our findings show that BPB possesses both antisecretory and antiulcer effects. Further studies are required to determine the role of BPB in the prophylaxis and/or the treatment of gastrointestinal ulcer diseases.

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REFERENCES

6. Swarm RA, Ashley SW, Soybel DI, Ordway FS, Cheung LY. Protective effect of exogenous phospholipid on aspirin-

Table 2  Effect of BPB on ethanol induced changes in Alcian blue binding capacity, NP-SH levels and MPO activity in gastric mucosa of rats (mean ± SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alcian blue binding (mg/g tissue)</th>
<th>Non-protein sulphydryl (mmol/g tissue)</th>
<th>Myeloperoxidase activity (A/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>935 ± 60.5</td>
<td>4.26 ± 0.11</td>
<td>0.081 ± 0.019</td>
</tr>
<tr>
<td>Ethanol (EtOH) alone</td>
<td>688 ± 25.2</td>
<td>2.96 ± 0.22</td>
<td>0.974 ± 0.067</td>
</tr>
<tr>
<td>EtOH + BPB 5 mg/kg</td>
<td>847 ± 75.8</td>
<td>4.08 ± 0.12</td>
<td>0.123 ± 0.024</td>
</tr>
<tr>
<td>EtOH + BPB 15 mg/kg</td>
<td>820 ± 70.6</td>
<td>4.02 ± 0.24</td>
<td>0.186 ± 0.089</td>
</tr>
<tr>
<td>EtOH + BPB 45 mg/kg</td>
<td>927 ± 34.2</td>
<td>4.80 ± 0.19</td>
<td>0.067 ± 0.025</td>
</tr>
</tbody>
</table>

*p < 0.05 and **p < 0.01 vs control group; †p < 0.05, ‡p < 0.01 vs ETOH alone (Dunnett’s test).


Al Moutaery AR, Tariq M. Effect of quinacrine, a phospholipase A2 inhibitor on stress and chemically induced gastroduodenal ulcers. Digestion 1997; 58: 129-137.


49 Kuchkina NV, Orlov SN, Chuchalin AG. The role of phospholipase A2, 5-lipoxygenase, and cyclooxygenase in activation of human neutrophil “oxygen burst”: the modulating effect of osmolality of the medium. Biokhimiia 1994; 59: 1034-1041

50 Laine L, Weinstein WM. Histology of alcoholic hemorrhagic “gastritis”: a prospective evaluation. Gastroenterology 1988; 94: 1254-1262


53 Miller TA, Li D, Kuo YJ, Schmidt KL, Shanbour LL. Nonprotein sulphydryl compounds in canine gastric mucosa: effects of PGE2 and ethanol. Am J Physiol 1985; 249: G137-G144


60 Kosower NS, Kosower EM. The glutathione status of cells. Int Rev Cytol 1978; 54: 109-160

S- Editor Pan BR L- Editor Zhu LH E- Editor Ma WH