

15-PGDH inhibitors: the antiulcer effects of carbenoxolone, pioglitazone and verapamil in indomethacin induced peptic ulcer rats

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Abstract. – BACKGROUND AND AIM: 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is the enzyme responsible for prostaglandins (PGs) metabolism. PGs have an important role in the protection of stomach mucosa against destructive stimuli. The aim of the present study is to investigate the inhibitory effect of carbenoxolone, pioglitazone and verapamil on 15-PGDH enzyme.

MATERIALS AND METHODS: The experiments were carried out in the Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt from May 2011 to August 2011. Adult male albino rats were fasted for 18 hours before administration of high dose of indomethacin (30 mg/kg, p.o.), except for the negative control group which received saline only, followed by pyloric ligation to induce acute gastric ulcers. The rats were pretreated orally with saline, pioglitazone (20 mg/kg), verapamil (25 mg/kg), carbenoxolone (30 mg/kg) or their combinations 30 minutes before indomethacin. The rats were sacrificed after four hours of pyloric ligation. The effects of the previous treatments on the ulcer index (U), the microscopic appearance of gastric mucosa, the gastric acid output, the gastric barrier mucus content, and 15-PGDH enzyme activity were determined.

RESULTS: Indomethacin resulted in severe ulceration and increased gastric acid output ($p < 0.05$) compared to negative control. The rats pretreated with carbenoxolone, pioglitazone, verapamil had reduced ulcer index, gastric acid output and 15-PGDH activity ($p < 0.05$) compared to either indomethacin group or the negative control group. Individual treatments with carbenoxolone, pioglitazone or verapamil increased gastric barrier mucus ($p < 0.05$) compared to either indomethacin group or the negative control group. The combinations of verapamil with either carbenoxolone or pioglitazone caused further reduction in ulcer index, gastric acid output and 15-PGDH activity ($p < 0.05$), while causing further increase in gastric barrier mucus ($p < 0.05$) compared to their respective individual treatment group.

CONCLUSIONS: The antiulcer properties of pioglitazone and verapamil are, in part, consequences of their inhibitory effect on the enzyme 15-PGDH, responsible for PGs degradation, and the resultant prolongation of PGE_2 biological activity in rat stomach mucosa.

Key Words:

15-hydroxyprostaglandin dehydrogenase (15-PGDH), Carbenoxolone, Prostaglandins (PGs), Pioglitazone, Ulcer, Verapamil.

Introduction

The non-selective nonsteroidal anti-inflammatory drugs (NSAIDs), including indomethacin, induce peptic ulcers in humans and experimental animal models¹⁻⁴. The ability of NSAIDs to inhibit the synthesis of PGs in the gastrointestinal tract (GIT) is believed to be the reason behind their harmful effects on stomach and duodenum⁵⁻⁷. PGs are a group of endogenously produced chemical transmitters working as autocrine or paracrine hormones⁸. They are potent chemical mediators found in picog/g tissue weight which are produced and metabolized within the same tissue and any remaining fractions that reach the systemic circulation are metabolised rapidly with plasma half-life of few minutes⁹.

Prostaglandins are synthesized from arachidonic acid by the action of cyclo-oxygenase enzymes (COX) which are either constitutional or inducible. The constitutional COX enzymes (e.g. COX-1) are responsible for production of PGs critical to the maintenance of normal physiological functions, including gastric mucosal integrity. The inducible COX enzymes (e.g. COX-2) are responsible for the production of PGs that mediate pain and inflammation. The therapeutic effects of NSAIDs are largely dependent on COX-2 inhibition, whereas undesirable effects on GIT including an increased risk of gastric ulceration and GIT bleeding are bound to COX-1 blockade¹⁰. Therefore, agents that selectively inhibit COX-2 over COX-1 were developed for the treatment of inflammation in order to avoid the harmful gastrointestinal effects caused by COX-1

blockade¹¹. However, the evidence showed that some COX-2 selective inhibitors resulted in mortalities, related to cardiac side effects, which led to their withdrawal by the manufacturers^{12,13}.

These findings about COX-2 inhibitors switched the focus of the scientists towards inventing more potent Proton Pump Inhibitors (PPIs) to be co-administered with non-selective NSAIDs in order to avoid their harmful effects on GIT and to investigate the different theoretical mechanisms underlying mucosal injury and repair¹⁴⁻¹⁸. Among the most accepted mechanisms is the concept of cytoprotection which has received a great deal of attention since 1979¹⁸. The concept of gastric cytoprotection described exogenous PGs mediated pathway as the major defense mechanism against harmful stimuli in non-antisecretory doses. Adaptive cytoprotection described endogenous PGs as the key substance responsible for mucosal protection. Restitution is another term that describes the ability of PGs to cause rapid epithelialization. Relying on these concepts, the antiulcer effects of some drugs were attributed to their ability to increase levels of PGs in gastric tissues, which are largely controlled by a balance between PGs producing and metabolizing enzymes¹⁹.

Prostaglandins are metabolized by the action of the catabolising enzymes, the 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and the 13-keto-prostaglandin reductase enzymes²⁰. 15-PGDH is a cytoplasmic enzyme that catalyzes the reversible oxidation/reduction of PGs at C-15²¹. Two isoenzymes were identified and characterized, the NAD⁺ dependent and the NADP⁺ dependent²². The predominant enzyme form in the gastrointestinal tract is the NAD⁺ dependent form²³. 15-PGDH catalyzes the first step in the deactivation of PGs. It converts PGE₂, PGF₂α and PGI₂ to their keto forms which have reduced biological activity^{21,24}.

Many drugs as well as many factors were found to reduce the 15-PGDH activity with subsequent increase in tissue PGs levels^{25,26}. Carbenoxolone is a drug used for the treatment of peptic ulcer that has antisecretory and mucus secretory properties²⁷⁻²⁹. The drug has been known to increase PGs levels by inhibiting the mucosal 15-PGDH and prostaglandin-13-reductase³⁰. More recent studies on 15-PGDH proved the ability of thiazolidinediones as a group of chemical compounds to reduce the enzyme activity^{31,32}. Pioglitazone, which belongs to this group, showed an ability to protect against stress, ethanol, acetic acid and indomethacin induced ulcers in rat models. It was reported to increase PGs levels in rat gastric mucosa

in some of these models³³. Concomitant to these findings about pioglitazone, a possible role of intracellular calcium depletion on 15-PGDH activity was elucidated³⁴. Previous studies have shown that calcium channel blockers, including verapamil, have the ability to reduce gastric acid secretion, increase mucus production and protect against ulcer³⁵⁻³⁹. Verapamil, like carbenoxolone and pioglitazone, has shown ability to increase PGs levels⁴⁰.

The present study hypothesizes that pioglitazone and verapamil would have inhibitory effects on 15-PGDH. These postulations were made by the analysis of previous studies^{31,32,34}. The present study investigates the hypothesized inhibitory effect of pioglitazone and verapamil on 15-PGDH activity in comparison to carbenoxolone, studies the effect of intracellular calcium depletion, induced by verapamil, on the extent of inhibition of 15-PGDH enzyme caused by either carbenoxolone or pioglitazone, and determines the effect of these drugs on ulcer index, gastric acid output, and gastric barrier mucus.

Materials and Methods

Animals

Adult male albino rats (160±10 g National Research Center, Cairo, Egypt) were used in the present study. They were housed in stainless steel cages with free access to food and water. The animals were habituated to the experimental conditions ten days before conduction of the experiment. They were maintained under controlled laboratory conditions of normal light-dark cycle, room temperature (25±3°C) and humidity (60%±10%). All animal procedures were approved by Department of Pharmacology and Toxicology of Faculty of Pharmacy, Suez Canal University (Ismailia, Egypt).

Chemicals and Drugs

Indomethacin, sterile saline (Nile Pharmaceuticals, Cairo, Egypt); pioglitazone (Medical Union Pharmaceuticals, Ismailia, Egypt); carbenoxolone, verapamil (MP Biochemicals, Solon, OH, USA); thiopental sodium (EPICO, Cairo, Egypt); NaOH, HCl, MgCl₂ (Elgomhoria, Cairo, Egypt); alcian blue 8GX, dithiothreitol, NAD⁺ (Sigma Aldrich, Saint Louis, MO, USA); sucrose, sodium acetate (El-Nasr Chemicals, Cairo, Egypt); Aerosol IB-45 solution (sodium diisobutyl sulfosuccinate 40% w/v) (CYTEC Industries, Woodland Park, NJ, USA); PGE₂,

prostaglandin E metabolite enzyme immunoassay kit (PGEM EIA kit) (Cayman Chemicals, Ann Arbor, MI, USA).

Induction of Ulcer and Treatment Groups

The rats were randomly assigned to seven groups (n=10; each). Gastric ulcers were induced by administration of high oral dose of indomethacin 30 mg/kg⁴¹. The animals were fasted 18 hours and allowed free access to water before administration of drugs. Group I, which served as a negative control, was given 0.5 ml saline orally. Group II (Indo), served as a positive control, and was given only indomethacin. Groups III, IV, V, VI and VII were given oral pioglitazone 20 mg/kg, verapamil 25 mg/kg, carbenoxolone 30 mg/kg, pioglitazone 20 mg/kg *plus* verapamil 25 mg/kg, and carbenoxolone 30 mg/kg *plus* verapamil 25 mg/kg respectively, 30 minutes before administration of indomethacin^{33,37,42}. The animals were then deprived of access to water for two hours, anaesthetized with 4 mg/kg thiopental sodium intraperitoneally, their abdomens were opened and the pylori were ligated according to method of Shay et al⁴³. They were sutured and injected with 10 ml saline subcutaneous and left to recover from anaesthesia. Animal deprivation of food and water was continued for another four hours; then they were euthanized and their stomachs were removed^{27,43,44}. The stomachs' contents were collected and stomachs were rinsed with cold saline solution, placed on ice for macroscopic examination, and then cut into several sections for different assays. The unused portions at the time of sacrifice were frozen immediately in -80°C for 15-PGDH activity assay. The following assays were then performed:

Macroscopic Examination (Ulcer Index)

The gastric lesions were counted, and an ulcer index (U_i) was calculated for each animal as follows:

$$U_i = (n \text{ lesion I}) + (n \text{ lesion II}) 2 + (n \text{ lesion III}) 3$$

Where:

- I = Presence of oedema, hyperaemia or petechiae (minor, submucosal, punctiform haemorrhages).
- II = Presence of submucosal, hemorrhagic lesions with small erosions.
- III = Presence of deep ulcer with erosions and invasive lesions⁴⁵.

The rats that did not show apparent stomach lesions or discoloration were assigned one point for U_i .

Determination of Gastric Acid Output

The contents of the stomachs were collected. The volume of the gastric juice for each animal was measured and a portion of 1 ml was titrated against 0.01 N NaOH to pH 7.0 using pH meter (OAKTON Instruments, Vernon Hills, IL, USA). The gastric acid output was determined in $\mu\text{Eq/hr}$ using the following equation^{46,47}.

Gastric acid output/Hour

$$\mu\text{Eq/hr} = \frac{0.01 \times \text{Vol. NaOH (ml)} \times \text{Vol. Gastric acid collected in 4 hours (ml)} \times 100}{4}$$

Determination of Gastric Barrier Mucus

The acidic mucus was measured *in situ* according to method of Corne et al⁴⁸ with a modification according to the original procedure developed by Whiteman⁴⁹. Stomach sections were weighed and immersed for two hours in 0.05% alcian blue 8GX dissolved in 50 mM MgCl₂ solution containing 58mM sucrose and buffered with 50 mM sodium acetate adjusted to pH 5.8 using HCl. The sections were immersed twice, 15 min and 45 min each, in 50 mM MgCl₂ solution containing 61mM sucrose and buffered with 50 mM sodium adjusted to pH 5.8 with HCl. The sections were destained using Aerosol IB-45 solution (sodium diisobutyl sulfosuccinate 40% w/v). The optical density was measured at 605 nm using a spectrophotometer (Shimadzu 1601, Shimadzu, Kyoto, Japan)^{48,49}.

Determination of 15-PGDH Activity

The activity of type 1 NAD⁺-dependent 15-PGDH enzyme was determined using an enzyme assay described in detail and characterized for use with intrauterine tissues⁵⁰. Briefly, central portions of frozen stomachs were weighed and placed in a metabolism buffer (0.1 mol PBS: phosphate buffered saline/L containing 2 mmol dithiothreitol/L; 50 mg tissue/ml buffer; 4°C). The tissues were homogenized (Polytron, Kinematica, Luzern, Swiss), each homogenate was centrifuged at 10 000 g for two min, diluted 1:20 with metabolism buffer and incubated with 1 mmol NAD⁺/L, 25 ng PGE₂ substrate in excess at 37°C for 15 min. The reaction was stopped by placing the homogenates on ice. The samples were purified using SPE cartridges (Waters, Milford, MA, USA). Prostaglandin E metabolite (PGEM) was determined using specific EIA kit. The results were expressed as pg PGEM/mg/min⁵¹.

Statistical Analysis

The data were expressed as the mean of six-to-eight experiments $\pm$ SD. The comparison of data for ulcer index was carried out using Kruskal-Wallis one-way analysis of variance (ANOVA) followed by Mann-Whitney *U* for comparison between two independent groups. The comparisons of data for other assays were carried out using ANOVA followed by Tukey HSD Multiple Comparisons Test. All analyses utilized SPSS 17.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA). A probability level of less than 0.05 was accepted as statistically significant.

Results

Macroscopic Examination

Indomethacin control group had higher ulcer index compared to negative control (41.63 ± 2.44 , $p < 0.001$). The groups treated with pioglitazone (P), verapamil (V), carbenoxolone (X), verapamil *plus* pioglitazone (VP), and verapamil *plus* carbenoxolone (VX) had lower ulcer indices compared to either indomethacin control or the negative control groups (5.00 ± 0.76 , 3.14 ± 0.38 , 3.75 ± 0.88 , 1.25 ± 0.46 , 1.63 ± 0.52 , $p < 0.001$, re-

spectively). The combinations' groups [i.e. verapamil *plus* either pioglitazone (VP) or carbenoxolone (VX)] showed reductions in ulcer indices compared to their respective individual treatments' groups ($p < 0.001$), (Figure 1, Table I).

Histopathological Examination

The sections obtained from indomethacin group showed several injuries with loss of normal morphology at different sites of mucosal epithelium. The inspection of lesions on higher magnification ($\times 1000$) revealed the existence of lymphocytic infiltration at different layers of mucosa including epithelium and lamina propria. The stomachs' sections of pioglitazone, verapamil, and carbenoxolone groups showed thickened, well defined mucosa. The sections obtained from verapamil *plus* pioglitazone and verapamil *plus* carbenoxolone groups showed greater mucosal thickening and higher mucus content within mucous and goblet cells and inside stomach glands. These sections showed enhanced epithelialization close to the uppermost mucosal layer and inside mucosal pits (Figure 2).

Gastric Acid Output

Indomethacin increased gastric acid output when compared to the negative control

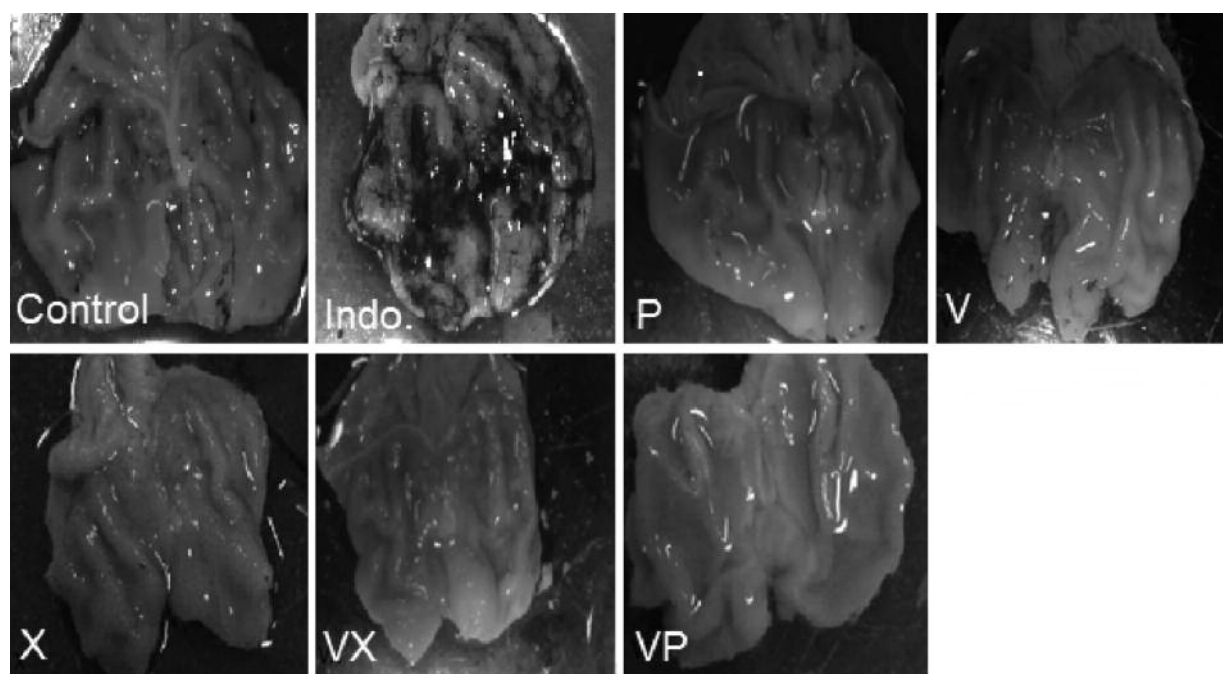


Figure 1. Photographs show gross morphology of stomachs from treatment, indomethacin or control groups (Digital JVC $\times 800$). Indo: indomethacin shows severe erosions, Control: shows submucosal small erosions, P: pioglitazone shows hyperaemia, V: verapamil and X: carbenoxolone show few petechiae, VP: verapamil+pioglitazone and VX: verapamil+carbenoxolone show normal appearance of mucosa no lesions or discoloration.

Table I. Effect of carbenoxolone, pioglitazone and verapamil on Ulcer index (U_i) in indomethacin induced peptic ulcer rats.

Groups	Ulcer index mean $\pm$ SD
Control	18.50 $\pm$ 1.77
Indomethacin	41.63 $\pm$ 2.44*
Pioglitazone+Indomethacin (P)	5.00 $\pm$ 0.76* [†]
Verapamil + Indomethacin (V)	3.14 $\pm$ 0.38* ^{†,‡}
Carbenoxolone + Indomethacin (X)	3.75 $\pm$ 0.88* ^{†,‡}
Verapamil + Pioglitazone + Indomethacin (VP)	1.25 $\pm$ 0.46* ^{†,‡,§}
Verapamil + Carbenoxolone + Indomethacin (VX)	1.63 $\pm$ 0.52* ^{†,§,#}

Treatments were given to rats 30 minutes before administration of indomethacin. Pylori were ligated under anaesthesia after two hours. Control was subjected to pylorus ligation only. All rats were sacrificed after four hours of pyloric ligation. *^{†,‡,§,#} $p < 0.05$ vs. control, indomethacin, P, V and X respectively using Kruskal Wallis one-way ANOVA followed by Mann-Whitney U test for two independent groups comparison.

(191.88 $\pm$ 12.79 $p < 0.001$). Pioglitazone, verapamil, carbenoxolone, verapamil *plus* pioglitazone and verapamil *plus* carbenoxolone groups showed decreased gastric acid output per hour compared to either indomethacin group or the negative control group (86.00 $\pm$ 5.83, 76.71 $\pm$ 7.01, 77.75 $\pm$ 5.47, 39.67 $\pm$ 7.99, 46.28 $\pm$ 7.25 μ Eq/hr, $p < 0.001$, respectively). The combinations produced greater

reduction in gastric acid output when compared to their respective individual treatments' groups $p < 0.001$ (Figure 3).

Gastric Barrier Mucus

Pioglitazone, verapamil, carbenoxolone, verapamil *plus* pioglitazone and verapamil *plus* carbenoxolone groups showed an increase in alcian blue extracted from stomach sections compared to either indomethacin control or the negative control groups (656.86 $\pm$ 45.88, 760.00 $\pm$ 34.64, 784.17 $\pm$ 37.47, 908.42 $\pm$ 73.40, 910.13 $\pm$ 49.51 μ g (alcian blue)/g (wet stomach tissue), $p < 0.001$, respectively). The combinations increased mucus production when compared to their respective individual treatments' groups ($p < 0.01$). The increase in mucus secretion by either verapamil or carbenoxolone was higher ($p < 0.01$) than the increase caused by pioglitazone (Figure 4).

15-PGDH Activity

Indomethacin decreased 15-PGDH activity compared to negative control (57.67 $\pm$ 5.79 pg/mg/min, $p < 0.05$). Pioglitazone, verapamil, carbenoxolone, verapamil *plus* pioglitazone and verapamil *plus* carbenoxolone showed decreased PGDH activity compared to indomethacin or the negative control groups (29.07 $\pm$ 1.54, 24.17 $\pm$ 1.30, 17.22 $\pm$ 2.64, 10.11 $\pm$ 2.30, 6.32 $\pm$ 0.77 pg/mg/min, $p < 0.001$, respectively). The combinations' groups

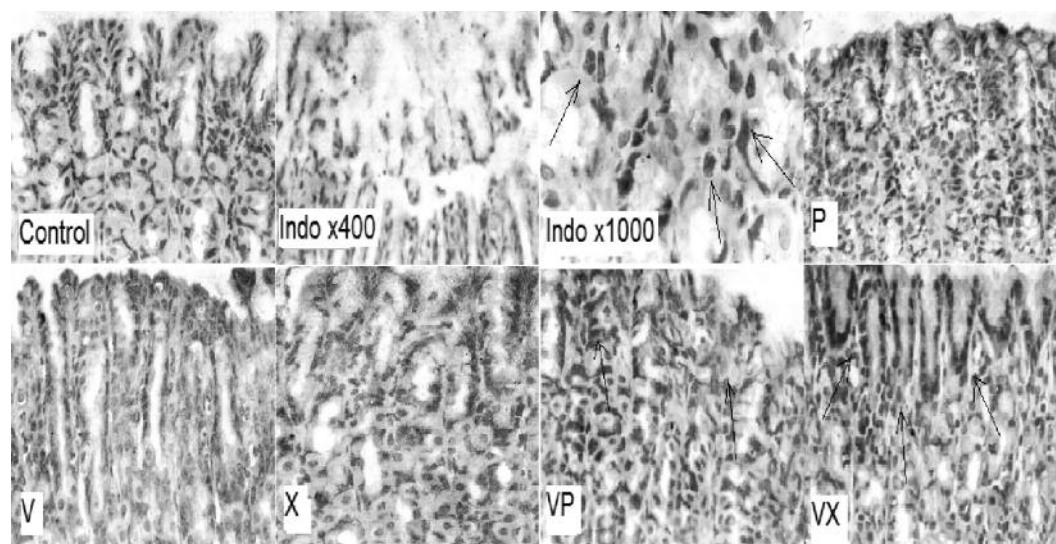


Figure 2. Photomicrographs for gastric mucosal transverse sections stained with H&E. P: Pioglitazone+Indomethacin, V: Verapamil+Indomethacin, X: Carbenoxolone+Indomethacin, VP: Verapamil+Pioglitazone+Indomethacin and VX: Verapamil+Carbenoxolone+Indomethacin. P, V, X show: normal appearance of mucosa without shedding or loss of morphology. VP, VX show increased mucus content in goblet cells and inside glands, increase in mucosal volume, increased mucosal thickness and enhancement of epithelial proliferation (arrows). Indo $\times$ 400: Indomethacin magnification 400 shows shedding of epithelium and desquamation. Indo $\times$ 1000: Indomethacin magnification 1000 shows Lymphocytic infiltration (arrows).

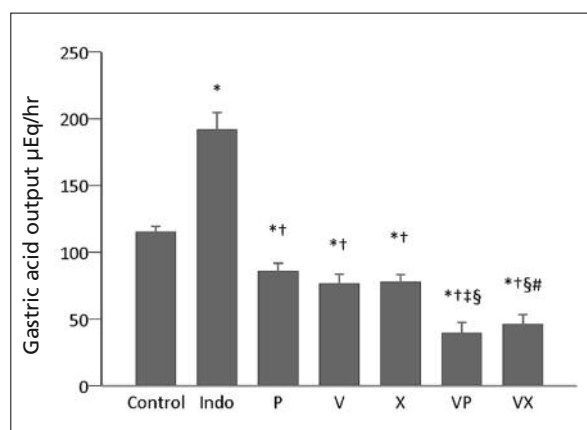


Figure 3. Gastric acid output $\mu\text{Eq/hr}$ (mean $\pm$ SD). Indo: Indomethacin, P: Pioglitazone+Indomethacin, X: Carbenoxolone+Indomethacin, V: Verapamil+Indomethacin, VX: Verapamil+Carbenoxolone+Indomethacin and VP: Verapamil+Pioglitazone+Indomethacin. *†‡§# $p < 0.05$ vs. Control, Indo, P, V, X respectively using one-way ANOVA followed by Tukey HSD Multiple Comparisons Test.

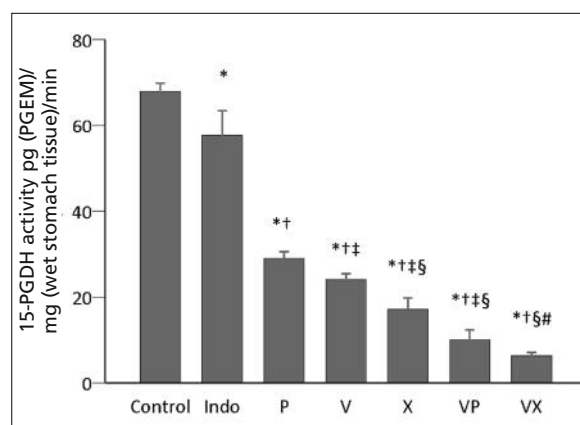


Figure 5. 15-PGDH activity pg/mg/min (mean $\pm$ SD). Indo: Indomethacin, P: Pioglitazone+Indomethacin, X: Carbenoxolone+Indomethacin, V: Verapamil+Indomethacin, VX: Verapamil+Carbenoxolone+Indomethacin and VP: Verapamil+Pioglitazone+Indomethacin. *†‡§# $p < 0.05$ vs. Control, Indo, P, V, X respectively using one-way ANOVA followed by Tukey HSD Multiple Comparisons Test.

showed reduced enzyme activity compared to their respective individual treatments' groups $p < 0.05$ (Figure 5).

Discussion

Prostaglandins are believed to have potent cytoprotective effects on the gastric glandular mucosal cells against ulcer induction by indomethacin. The

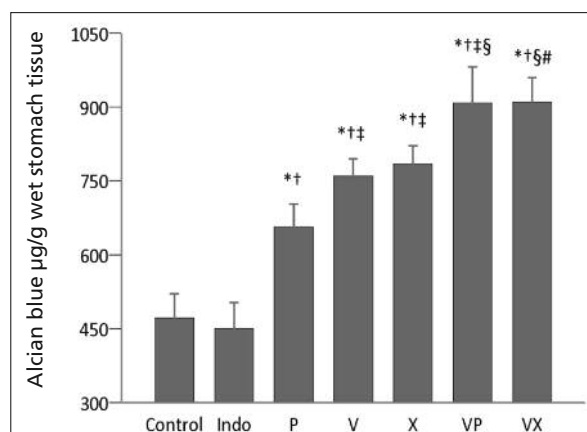


Figure 4. Alcian blue $\mu\text{g/g}$ wet tissue weight (mean $\pm$ SD). Indo: Indomethacin, P: Pioglitazone+Indomethacin, X: Carbenoxolone+Indomethacin, V: Verapamil+Indomethacin, VX: Verapamil+Carbenoxolone+Indomethacin and VP: Verapamil+Pioglitazone+Indomethacin. *†‡§# $p < 0.05$ vs. Control, Indo, P, V, X respectively using one-way ANOVA followed by Tukey HSD Multiple Comparisons Test.

glandular mucosa is the main protective structure which maintains stomach integrity¹⁹.

The present study hypothesizes that “the drugs that can inhibit PGs catabolising enzyme 15-PGDH would protect against indomethacin induced ulceration as a result of the accumulation of gastroprotective PGE_2 in gastric tissues. The accumulation of this gastroprotective PG is a consequence of blocking the metabolism of PGs by 15-PGDH inhibitor drugs”. Carbenoxolone, which has a documented enzyme inhibitory action, and pioglitazone a possible 15-PGDH enzyme inhibitor, were chosen to determine their effects on 15-PGDH activity in the stomach of rat ulcer model^{30,31}.

The second assumption adopted by the present study is that “the gastroprotective effects of calcium channel blockers” are the fruits of the accumulation of PGs in gastric mucosal tissues resulting from 15-PGDH inhibition. The inhibition of 15-PGDH is a direct consequence of depletion of intracellular calcium caused by these drugs. This relationship between 15-PGDH inhibition and the reduction of intracellular calcium stores has been reported before in a previous study³⁴. However, the effect of 15-PGDH inhibition on PGs levels in stomach mucosa has not been studied before. Verapamil was selected among other calcium channel blockers due to previous reports which have demonstrated that it has gastroprotective effects³⁶⁻³⁸.

An ulcer model that combines indomethacin and pylorus ligation was employed in the present study to determine the effect of the three drugs,

carbenoxolone, pioglitazone and verapamil, on gastric acid output⁴⁴. In addition, we measured the severity of the ulceration induced by indomethacin macroscopically and microscopically in order to assess the ability of the three gastroprotective drugs to reduce indomethacin induced ulceration. Similarly, the effect of the drugs on the stimulation of gastric mucus secretion was measured as an important mucosal defense factor and one of the components of PGs mediated gastroprotection. Finally, in order to verify the hypothesis proposed by this research, we measured the activity of 15-PGDH enzyme in the stomach tissues.

The results of the determination of 15-PGDH activity support what the present study hypothesizes about the inhibitory effect of verapamil and pioglitazone and confirms the previously reported inhibitory effects of carbenoxolone on the 15-PGDH enzyme^{30-32,34}. The present study suggests that the decrease in 15-PGDH enzyme activity with the subsequent prolongation of the biological activity of the locally generated PGs contributes to the protective and ulcer-healing activity of pioglitazone and verapamil. A similar mechanism has previously been suggested as a possible mechanism for gastroprotection mediated by carbenoxolone⁵². Moreover, the results of 15-PGDH activity determination from the groups pretreated with combinations of carbenoxolone or pioglitazone with verapamil show a greater reduction in enzyme activity. Whether the effect of adding verapamil to pioglitazone or carbenoxolone was additive or synergistic could not be identified in the present study. However, these findings support what we have hypothesized about the effect of reducing intracellular calcium on the enzyme activity.

The findings of macroscopic examination reveal that pyloric ligation alone can produce visible gastric ulcers resulting from accumulation of gastric secretion; this is consistent with previous works¹. These findings show that indomethacin administration can further aggravate the mucosal damage and increase the ulcers index. It is known that indomethacin can induce ulcer in rats by inhibiting PGs biosynthesis and this effect is believed to be potentiated by the gastric acid^{53,54}. The pretreatment with carbenoxolone, pioglitazone, verapamil, or their combinations resulted in a marked decrease in severity and number of lesions produced by indomethacin. The antiulcer effect of the three drugs was previously reported by several authors^{33,36,55}. Moreover, animals treated with drug combinations were greatly or fully

protected against ulcer induction by indomethacin and their stomachs showed fewer and less severe lesions or no lesions at all. These findings suggest that these drugs have an additive protective effect with regard to stomach mucosa.

The macroscopic findings are supported by the microscopic examination of stomach sections. Mucosal shedding, desquamation and lymphocytic infiltration were evident in stomachs' transverse sections derived from indomethacin control group. The microscopic picture was different in the sections obtained from the groups pretreated with carbenoxolone, verapamil or pioglitazone which showed intact mucosal layers with no apparent lesions or deformities. Furthermore, the transverse section obtained from the groups treated with combinations showed enhanced epithelisation at surface and mucosal pits. The mucous cells in treatment groups showed enhanced proliferation and they were enriched with mucus content which was greater in sections obtained from combinations groups.

The determination of acidic mucin using alcian blue reveals that carbenoxolone, verapamil, pioglitazone, or their combinations have increased the acidic mucin production compared to indomethacin group or negative control groups. The findings on carbenoxolone are consistent with findings from a previous report that adopted the same technique²⁹. The effect of carbenoxolone on gastric mucus was previously attributed to its ability to increase PGs levels in stomach tissues⁵⁶. Similarly, our results show that verapamil has increased gastric barrier mucus compared to indomethacin or negative control groups. The stimulatory effect of verapamil on mucus secretion^{35,37} may be attributed to its previously reported ability to increase PGs levels⁵⁷. The authors of the present study attribute the ability of verapamil to increase mucus production to its ability to increase the levels of PGE₂ as a result of inhibition of 15-PGDH. Finally, pioglitazone increased gastric barrier mucus. To the best of our knowledge, this is the first report of such an effect in rat stomach. A recent work has investigated the effect of pioglitazone on mucus in mice lung. Pioglitazone has been reported to increase airway mucus production in mice. This effect has been explained by the ability of pioglitazone to increase the production of T-Helper 2 cells inflammatory cytokines in toluene diisocyanate induced airway inflammation⁵⁸. Moreover, other reports on pioglitazone showed that it can increase the levels of endogenous PGs in different ulcer models³³. We attribute the mucus secretory

properties of pioglitazone in the stomach to its ability to increase PGs rather than an effect mediated by inflammatory cytokines; the increase in PGE₂ concentrations may be due to the blockade of PGs metabolizing enzyme, 15-PGDH, caused by pioglitazone.

The findings of determination of gastric acid output reveal that indomethacin resulted in increased gastric acid output compared to the negative control. This effect is consistent with other data about the drug. It has been previously suggested that the inhibition of COX-1 with the resultant decrease in PGs levels is responsible for the increase in gastric acid secretion and ulcerogenic effects caused by indomethacin⁵⁹. Our research supports this assumption. The administration of carbenoxolone, pioglitazone, verapamil, or their combinations, 30 minutes before administering indomethacin, resulted in reducing gastric acid output to levels below that produced in either of the two control groups. The drugs' combinations further lowered the gastric acidity below that produced by individual treatments. Carbenoxolone has been known to decrease gastric acid secretion²⁷. This property was reported long before recognizing its ability to increase PGs levels in gastric tissues^{30,56}. It is believed that drugs that increase PGs levels can eventually decrease gastric acid output; this action of PGs is believed to be mediated via EP3 receptors in parietal cells^{23,30,60}. The present study endorses these referred findings and suggests that the antisecretory effect of carbenoxolone is a consequence of its ability to increase PGs. Moreover, the antisecretory effect of verapamil was reported at high doses exceeding or equal to 25 mg/kg. This effect is strongly believed to be a result of direct blockade of calcium channels found on enterochromaffin-like (ECL) cells resulting in decreased histamine release from these cells. It is known that histamine stimulates gastric acid secretion. This direct effect on ECL cells can be one cause behind the ability of verapamil to decrease gastric acid output³⁹. The present study suggests that the ability of verapamil to increase PGs levels can be a second important cause, especially when we know that PGs can also reduce histamine release from ECL cells⁶¹.

In the present work, pioglitazone has produced similar effects to that caused by carbenoxolone on gastric acid output. The previous reports about the effect of pioglitazone on gastric acid output were conflicting. Two recent papers on the effect of pioglitazone on gastric acid output have reported opposing results^{62,63}. The first study, performed on

gastric glands isolated from mice that have been treated with pioglitazone⁶², showed that prior treatment with pioglitazone resulted in increase in the gastric acid secretion stimulated by ammonium pulse. The authors concluded that pioglitazone increases gastric acid output from parietal cells due to stimulation of serum and glucocorticoid inducible kinase (SGK1) gene expression in gastric glands resulting in stimulation of KCNQ1K+ channels. The second study reported that pioglitazone administration in a dose of 10 mg/kg for three days can decrease gastric acid secretion in rats. The authors have related this effect to the ability of pioglitazone to increase PGs levels⁶³. Our findings agree with the findings of the second study. An explanation for the conflicting conclusions is the differences in methodologies and in treatment periods. A careful review of these two reports can provide some insight about these conflicts. The first study was performed on isolated gastric glands, thus the effect of pioglitazone on stomach glands was evaluated separately from the adjacent structures. While PGs are abundant in stomach layers other than mucosa (i.e. muscularis mucosa, submucosa and serosa), this study on isolated mucosal glands neglected the effect of local circulation which can convey higher concentrations of PGs from adjacent tissues⁶⁴.

Conclusions

Prostaglandins metabolizing and synthesizing enzymes are equally important in GIT and the balance between production and metabolism is the determinant of PGs levels in gastric tissues. The inhibitory effect that carbenoxolone, verapamil and pioglitazone exert on 15-PGDH is in part responsible for their ability to increase PGs levels in rat stomach and is one important cause of their gastroprotective effects.

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Conflict of Interest

None.

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