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Pantoprazole reduces vascular relaxation *in-vitro* and *ex-vivo* and interferes with blood coagulation in an animal model

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ABSTRACT

Background and aims: Proton pump inhibitors (PPIs) are effective antagonists of gastric acid secretion used to treat a number of gastro-esophageal disorders. The present study investigated the effect of Pantoprazole on vascular relaxation *in-vitro* and *ex-vivo* and its effect on blood coagulation in an animal model.

Main methods: Isolated mouse arterial rings were pre-contracted *in-vitro* with phenylephrine and concentration–response curves to the acetylcholine relaxing effect were constructed in the presence of escalating concentrations of pantoprazole. In another set of experiments, male albino mice weighing ~25 g were administered a daily dose of pantoprazole (0.4 mg by oral gavage) for four consecutive weeks; a vehicle control group was run in parallel. At the end of the treatment period, thoracic aorta was isolated for the assessment of vascular function *ex-vivo*. Blood samples were also collected to evaluate the effect of chronic pantoprazole therapy on coagulation parameters, namely, prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Key findings: Vascular responsiveness to acetylcholine demonstrated a reduced relaxation of the arterial ring from baseline in the presence of different concentrations of pantoprazole (1 μM: 54.69 ± 1.42%, 10 μM: 34.64 ± 0.90% and 100 μM: 31.50 ± 0.67% vs. control 74.39 ± 1.426%, $p < 0.001$). Furthermore, acetylcholine-induced relaxation of the aorta was significantly diminished after four weeks of administrating pantoprazole to mice (37.12 ± 2.50%) compared with the control group (72.47 ± 1.68%, $p < 0.001$). This, however, wasn't accompanied by significant changes in the phenylephrine-induced vasoconstriction. Animals that received pantoprazole daily for four weeks also exhibited increased blood coagulation time in comparison to the vehicle control group (PT 45.30 ± 3.52 s *vs.* 15.30 ± 0.70 s, $p < 0.05$; aPTT 96.1 ± 4.62 s vs. 48 ± 1.97 s, $p < 0.05$, respectively).

Significance: The results of the present investigation suggest that pantoprazole reduces arterial relaxation and interferes with blood coagulation. Additional studies are warranted to assess the clinical implications of such observations.

1. Introduction

Proton pump inhibitors (PPIs) are effective antagonists of gastric acid secretion used to treat a number of gastro-esophageal disorders including dyspepsia, gastroesophageal reflux disease (GERD) and peptic ulcer disease [1]. They are the preferred drugs of choice over the histamine H_2-receptor antagonist due to their superiority to decrease gastric acid secretions [2,3]. Patients receiving anti-platelets therapy (e.g., aspirin, clopidogrel) and/or anticoagulant drugs such as warfarin for acute coronary syndrome or percutaneous coronary intervention are frequently prescribed PPIs to offer a gastro-protective effect against gastrointestinal hemorrhage Gillard; Lozano [4,5]

The US FDA issued warning against the concomitant use of clopidogrel with omeprazole and esomeprazole has become a continued topic for discussion in the literature [6]. A systemic review reported concomitant use of PPIs with [5] anti-platelets led to adverse outcomes for major adverse cardiovascular events (MACE) and mortality [7]. This perhaps is due to a decrease in the anti-platelet efficacy of clopidogrel in presence of PPIs [8]. However, there is a varying degree of evidence regarding this association. Most of the studies have concentrated on omeprazole and lansoprazole; but very few conducted on other PPIs [9]. For example, a randomized trial showed that pantoprazole did not affect the pharmacodynamics and the pharmacokinetics of warfarin [9]. A review on the pharmacokinetic interactions of PPIs suggested

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that pantoprazole has low potential to interact with other drugs [10]. When pantoprazole was used with clopidogrel, adverse events did not increase [11]. According to expert opinion, anti-platelet drugs should be used with caution along with PPIs [12]. Recently, there has been growing concern for the use of PPIs and their intrinsic association with cardiovascular events [13]. According to a study by Shah and colleagues, there is an association between PPIs exposure with the risk of myocardial infarction (MI) [13]. The use of PPIs has been viewed as an independent risk factor for MI [1]. Furthermore, the use of PPIs was linked to increased incidences of heart failure (HF) and death in coronary artery disease (CAD) patients [3]. It is also known that pantoprazole may exert negative inotropic effects on isolated myocardium from humans and rabbits [14]. Presently, there is no available data that has explored the inherent effect of pantoprazole on vascular function and blood coagulation. Therefore, the aim of the current study was to investigate the effects of pantoprazole on the vascular function and the blood coagulation time in an animal model both *in-vitro* and *ex-vivo*.

2. Methods

2.1. Chemicals and reagents

Phenylephrine and acetylcholine were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Pantoprazole was obtained from Taketa GmbH (Oranienburg, Germany). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) kits were obtained from Diagnostica Stago (Asnières sur seine, France).

2.2. Animals and conditions

Male BALB/c Albino mice weighing ~25 g were obtained from the College of Pharmacy Animal Care and Use Facility, King Saud University (Clearance No. 2117-EACC, 2017). Animals used in the study were maintained according to the recommendations of the "Guide for the Care and Use of Laboratory Animals" approved by the facility (NIH publications no. 80-23; 1996). They were housed in filter-top shoebox cages in a temperature and humidity-controlled room with a 12-h light/dark cycle and were allowed access to food and water *ad libitum* during the study except that the chow was removed 12 h prior to animal euthanization.

2.3. Vascular reactivity experiments

The effect of pantoprazole on vascular reactivity was studied by using isolated mouse thoracic aorta (n = 6). In brief, after sacrificing the animal, the aorta was promptly extracted and placed in Krebs–Henseleit physiological salt solution (control solution) of the following composition in mmol/L: NaCl, 129; $NaHCO_3$, 25; glucose, 5.5; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.17; EDTA, 0.025; pH 7.4 at 37 °C. The aorta was separated and freed of neighboring connective tissue, cut in segments of 2 mm length and using 40 μm diameter stainless steel wires, anchored on a wire myograph (620 M, Danish Myo Technology 'DMT', Aarhus, Denmark) for recording isometric contractile force. Each sample was equilibrated for 30 min in control solution, maintained at 37 °C and aerated with a carbogen gas mixture (5% CO_2 and 95% O_2). The normalized passive resting force and the corresponding diameter were then determined for each segment of aorta tissue from its own length-tension curve. Contractile responses were recorded by Power Lab Data Acquisition System (AD Instruments, Sydney, Australia) linked to a computer, installed with a Lab Chart software (Version 7). After stabilization and equilibration for 30 min in control solution, the preparations were exposed to 60 mM KCl solution to obtain a reference contraction. The rings were allowed to equilibrate for 40 min and then exposed to cumulatively increasing concentrations (10^{-10}–10^{-4} M) of phenylephrine. Once the contraction to phenylephrine reached steady state, cumulative concentrations of acetylcholine (10^{-10}–10^{-4} M) were added to the organ chamber. Relaxations to the cholinergic transmitter are expressed in percentage of the pre-existing contractile tone induced by phenylephrine in the presence of escalating concentrations of pantoprazole (i.e., 1, 10, and 100 μM). In another set of experiments, male albino mice (n = 6), weighing ~25 g, were treated with a daily dose of pantoprazole (0.4 mg by oral gavage, which is equivalent to 80 mg in a 70 kg man) for four consecutive weeks; a vehicle control group was run in parallel (n = 6). At the end of the treatment period, thoracic aorta was isolated from the animals for the assessment of vascular reactivity *ex-vivo* as described above.

2.4. Blood coagulation experiments

The effect of pantoprazole on blood coagulation measured by prothrombin time (PT) and activated partial thromboplastin time (aPTT) was investigated after chronic administration of pantoprazole (0.4 mg) or control vehicle (0.5% carboxy-methylcellulose) given by oral gavage daily for four weeks (n = 10). Blood samples were collected at the end of the experiment and plasma was separated by centrifugation of the whole blood at 10,000 rpm for 10 min. The test was performed by adding PT or aPTT reagents to plasma and measuring the time for clot formation according to manufacturer's instructions (Diagnostica Stago Asnières sur seine, France).

2.5. Statistical analysis

The results are presented as mean ± SEM. Myography data points representing preparations from six different mice were analyzed using one-way ANOVA and Tukey multiple comparison test. An un-paired *t*-test was carried out to determine the difference in PT and aPTT between the pantoprazole group and controls from ten different mice in each group. GraphPad Prism version 6 for Windows (San Diego, CA, USA) was used for performing all analyses. Significance was set at a p-value of less than 0.05.

3. Results

Vascular responsiveness to acetylcholine demonstrated a reduced relaxation of the arterial ring from baseline in the presence of different concentrations of pantoprazole (1 μM: 54.69 ± 1.42%, 10 μM: 34.64 ± 0.90% and 100 μM: 31.50 ± 0.67% vs. control 74.39 ± 1.426%, $p < 0.001$) (Fig. 1 and Table 1). Furthermore, acetylcholine-induced relaxation of the aorta was significantly diminished ex-vivo in the pantoprazole treated mice (37.12 ± 2.50%) compared with controls (72.47 ± 1.68%, $p < 0.001$). This was also associated with increased acetylcholine concentrations needed to relax the aorta in treated mice vs. controls ($logEC_{50}$: −7.15 ± 0.15 M *vs.* −5.91 ± 0.02 M, respectively, $p < 0.01$). There were no significant changes observed in the phenylephrine-induced vasoconstriction in all treatment groups (Fig. 1 and Table 2). Mice treated with pantoprazole daily for four weeks exhibited increased coagulation time measured by PT and aPTT in comparison to the vehicle control group (PT 45.30 ± 3.52 s vs. 15.30 ± 0.70 s, $p < 0.05$; aPTT 96.1 ± 4.62 s *vs.* 48 ± 1.97 s, $p < 0.05$, respectively) (Fig. 2).

4. Discussion

In this study we explored the effect of pantoprazole on the vascular function and blood coagulation in a mouse model *in-vitro* and *ex-vivo*. The findings showed that in the presence of pantoprazole at different concentrations there was a decrease in acetylcholine-induced vascular relaxation of the arterial ring. The current findings are similar to that of Ghebremariam and co-workers where omeprazole was used [15]. However, omeprazole and lansoprazole have been found to induce relaxation of isolated human internal mammary and radial arteries [16].