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Gastroprotective and anti-secretory mechanisms of 2-phenylquinoline, an alkaloid isolated from *Galipea longiflora*

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ABSTRACT

Background: We previously described the gastroprotective effect of 2-phenylquinoline (2-PQ), the main alkaloid isolated from the bark of *Galipea longiflora* (Rutaceae). However, despite the significant and promising results, the pharmacological mechanisms of the gastroprotection induced by 2-PQ have not been investigated.

Purpose: To evaluate the mechanisms underlying the gastroprotective effects of 2-PQ.

Study design: We used an *in vivo* mouse ulcer model and *in vitro* methodologies involving H^+/K^+-ATPase and L929 murine fibroblasts.

Methods: The gastroprotective activity of 2-PQ (10–100 mg/kg, orally, p.o) was assessed against gastric ulcer induced by 60% ethanol/0.03 M hydrochloric acid (HCl) in mice or that induced by indomethacin (80 mg/kg, p.o) in rats. The cytotoxicity was assessed in L929 murine fibroblasts. Ulcerated tissues were analyzed histologically, histochemically, and biochemically. The antisecretory activity of 2-PQ was evaluated *in vivo* and *in vitro*.

Results: 2-PQ showed no cytotoxicity, reduced the lesion area induced by ethanol/HCl (log half-maximal effective dose, $ED_{50} = 1.507$), and the histological evaluation supported these results. Furthermore, 2-PQ reduced indomethacin-induced gastric ulceration. The gastroprotection was accompanied by normalization of superoxide dismutase (SOD) and glutathione-*S*-transferase (GST) activity, an intense increase in reduced glutathione (GSH) levels, and reduction in lipid peroxide (LPO) and tumor necrosis factor (TNF)-α levels in the gastric mucosa. The antisecretory properties of 2-PQ were confirmed by the decreased volume and total acidity of the gastric juice, and it reduced histamine- or pentagastrin-stimulated gastric acid secretion. However, 2-PQ did not change the *in vitro* H^+/K^+-ATPase activity or the content of gastric-adhered mucous in mice. In addition, pretreatment with N-ethylmaleimide, NG-nitro-L-arginine methyl esters, yohimbine, or indomethacin reversed the gastroprotective effect of 2-PQ, suggesting nitric oxide, nonprotein sulfhydryl compounds, α-2-receptors, and prostaglandin were involved.

Conclusion: 2-PQ provides gastroprotection by reducing oxidative damage and inhibiting acid secretion mediated by histaminergic and gastrinergic regulatory pathways.

Introduction

Gastric ulcers are typically caused by an imbalance between protective factors (epithelial barrier, mucus secretion, blood flow, and cell regeneration secretion) and aggressive factors (acid-pepsin secretion and reactive oxygen species [ROS]) in the gastric mucosa (Hunt et al., 2015). Currently, the main pharmacological treatment for this disease is antisecretory drugs including, histamine

Abbreviations: 2-PQ, 2-phenylquinoline; ROS, reactive oxygen species; MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]; DMSO, Dimetilsufoxide; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; TLC, thin-layerchromatography; NMR, nuclear magnetic resonance; NCTC, murine fibrosarcoma L929; ALFAC, 85% ethanol, 10% formalin and 5% acetic acid solution; PAS, Periodic acid-Schiff; GSH, reducedglutathione; LOOH, lipidhydroperoxides; BHT, butylatedhydroxytoluene; EDTA, Ethylenediaminetetraaceticacid; H_2O_2, Hydrogen peroxide; GST, glutathione-S-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; DPPH, 2,2-diphenyl-1-picrylhydrazyl; MPO, myeloperoxidase; TMB, 3,3′,5,5′-tetramethylbenzidine; TNF, Tumor necrosisfactor; NEM, N-Ethylmaleimide; L-NAME, N-ω-nitro-L-argininemethylester.

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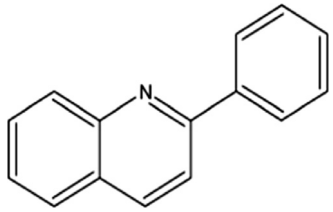

Fig. 1. 2-phenylquinoline (2-PQ).

type 2 receptor antagonists such as cimetidine and congeners, and irreversible proton pump inhibitors such as omeprazole and congeners. Although effective, long-term therapy with these drugs is associated with several side effects and poor gastric healing, leading to ulcer recurrence (Kangwan et al., 2014).

Galipea longiflora Krause [synonymn *Angostura longiflora* (Krause) Kallunki], a member of Rutaceae family of the Bolivian Amazon, is popularly known as Evanta and used in folk medicine by Amazonian ethnic groups (Bourdy et al., 2000). Previous studies in our continuing search for bioactive natural products revealed the gastroprotective effects of 2-phenylquinoline (2-PQ), an alkaloid isolated from *G. longiflora*, in rodents (Zanatta et al., 2009). However, the mechanisms of the gastroprotective effects of 2-PQ have not been investigated, and considering that understanding the actions of natural products is an important step in drug development, this study was designed to elucidate these gastroprotective mechanism of 2-PQ.

Materials and methods

Chemicals and drugs

The following substances were used: Alcian blue, Bovine serum albumin, Carbenoxolone, glutathione, indomethacin, MTT, omeprazole, 2,2-diphenyl-1-picrylhydrazyl, 5,5′-dithiobis (2-nitrobenzoic acid), 3,3′,5,5′-tetramethylbenzidine, pyrogallol, ranitidine and xylenol orange were purchased from Sigma-Aldrich®, St. Louis, MO, USA. Ascorbic acid, Ethanol, diethylether, ferricchloride, formaldehyde, hydrogen peroxide, magnesium chloride, methanol, N,N-dimethylformamide, sodium acetate, sodium carbonate, sucrose and trichloroacetic acid were purchased from Vetec®, Rio de Janeiro, RJ, Brazil. Dimetilsufoxide and N,N-dimethylformamide (DMSO) was obtained from Synth, Diadema, SP, Brazil. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Vitrocell®, Campinas, SP, Brazil and fetal bovine serum (FBS) from Gibco®, Gaithersburg, MD, USA. All reagents and drugs were prepared immediately before use. Water was processed using the Milli-Q system (Millipore®32, MA, USA). All other reagents and solvents were of analytical grade.

Plant material, extraction and isolation

The collection of plant material, and the extraction and isolation procedures used, have been previously described by Zanatta et al. (2009). Briefly, bark of *G. longiflora* was collected in the Bolivian province of Nor Yungas and deposited in the National Herbarium (La Paz, Bolivia) .The alkaloid fraction was obtained from this plant material as previously described (Gimenez et al., 2005).

1 g of alkaloid fraction was chromatographed on a silica-gel column and eluted with a gradient of ethyl acetate in n-hexane. The fractions yielded 0.55 g of 2-phenylquinoline (Fig. 1), identified by thin-layerchromatography and nuclear magnetic resonance spectral data in comparison with an authentic sample.

Cell culture and cell viability assay

Murine fibrosarcoma L929 (NCTC clone 929) from the Rio de Janeiro cell bank were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in 5% CO_2, with humidified atmosphere, at 37 °C. L929 cells (1×10^5) were cultured in 96-well plates (in triplicates) in the presence of vehicle (culture medium with 0.1% of DMSO), 10% DMSO (negative control) or *2-PQ* (0.1–100 µg/ml) at 37 °C for 24 h. Thereafter, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to cells and cell viability was measured as described by da Silva et al (2015).

Animals

Male Wistar rats (180–200 g) and male Swiss mice (25–30 g) were obtained from the Central Animal House of the Universidade do Vale do Itajaí (UNIVALI), Itajaí – SC. One adult albino rabbit (*Oryctolagus cuniculus*) (~2 kg) was used for gastric H^+,K^+-ATPase assay. The rodents were housed in standard cages ($n = 6$), at room temperature (25 ± 3 °C), with 12 h dark/12 h light cycles with access to pellet food (Biobase, Águas Frias/SC, Brazil) and water *ad libitum*. Twelve h before the experiments, the animals were placed on fasting with water *ad libitum* in cages with wide-mesh raised floors to prevent coprophagy. The experiments were authorized by Ethical Committee for Animal Care of the UNIVALI (CEUA: 38/14p) and were conducted in accordance with the "Principles of Laboratory Animal Care" (NIH Publication 85–23, 1985).

Antiulcer activity

Ethanol/HCl-induced gastric ulcer

The antiulcerogenic activity of 2-PQ was evaluated as described by Hara and Okabe (1985), with few modifications. The mice were divided into five groups ($n = 6$). The first group received vehicle (water plus 1% tween®, 10 ml/kg, p.o), the second group was treated with carbenoxolone (positive control, 200 mg/kg, p.o), and the other three groups received 2-PQ (10, 30 or 100 mg/kg, p.o). One h after this treatment, gastric lesions were induced by oral administration of 60% ethanol/ 0.03 M HCl (10 ml/kg). The animals were euthanized in a CO_2 atmosfere one h after ulcerogenic agent intake. The stomachs were removed and opened along the greater curvature, and the lesion area (mm^2) was measured using the software program EARP®.

Nonsteroidal anti-inflammatory drugs (NSAIDs)-induced gastric ulcer

The experiment was conducted in rats in accordance with Djahanguri (1969). The animals were divided into three groups ($n = 6$), and orally treated with vehicle (water°+ 1% tween®, 1 ml/kg), or carbenoxolone (200 mg/kg) or 2-PQ (30 mg/kg). One h after, all rats received oral administration of indomethacin (80 mg/kg) to induce gastric lesions. The rats were euthanized in a CO_2 atmosphere 6 h after indomethacin administration; the stomachs were removed and opened to evaluate the lesioned area (mm^2), as described above.

Histological and histochemical analysis

A portion of stomach tissue exposed to ethanol/HCl were fixed, dehydrated, embedded in paraffin, cut on 5 µm and stained by the hematoxylin and eosin (HE) method (Laine and Weinstein, 1988). For the determination of mucin, the method described by Mowry and Winkler (1956) was used, with few modifications. Periodic acid-Schiff (PAS)-stained mucin-like glycoprotein positive pixels were quantified using the software program ImageJ®, with magnification of 400×.