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Gastroprotective effects of goniothalamin against ethanol and indomethacin-induced gastric lesions in rats: Role of prostaglandins, nitric oxide and sulfhydryl compounds

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ABSTRACT

Goniothalamin (GTN), a styryl-lactone, is a secondary metabolite naturally found in its enantiomeric form (*R*) in plants of the genus *Goniothalamus* (Annonaceae). The antiproliferative activity against human tumor cell lines reported in several studies suggest that the α,β-unsaturated δ-lactone moiety emerges as a key Michael acceptor for cysteine residues or other nucleophilic biological molecules. Our group reported on the *in vivo* activity of (*R*)- and (*S*)-GTN as well as its racemic form (*rac*-GTN) in both Ehrlich solid tumor and carrageenan-induced paw edema in mice, without side effects in the effective doses. Despite the rich body of data on the *in vitro* GTN biological activity, much less is known about its *in vivo* pharmacological action. Herein we describe the gastroprotective activity of *rac*-GTN on chemical-induced gastric ulcers models in rats. GTN has a potent gastroprotective effect on ethanol-induced ulcers (effective $dose_{50}$ = 18 mg/kg) and this activity is dependent on sulfhydryl compounds and prostaglandins generation, but independent of nitric oxide (NO), gastric secretion and mucus production. We hypothesize that goniothalamin may act as a mild irritant, inducing the production of sulfhydryl compounds and prostaglandins, in a process known as adaptive cytoprotection. This hypothesis is supported by the fact that Michael acceptors are the most potent inducers of antioxidant response (as activation of Nrf2 pathway) through generation of mild oxidative stress and that gastroprotective activity of goniothalamin is inhibited after pre-treatment with NEM (*N*-ethylmaleimide) and NSAID (non-steroidal anti-inflammatory drugs), highlighting the importance of sulfhydryl compounds and prostaglandins on GTN activity.

1. Introduction

Peptic ulcer disease encompasses both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries [1], affecting each year 4 million people around the world [2]. Gastric mucosa is continuously exposed to damaging agents involved in the pathogenesis of gastric ulcers and the basis of this pathology is an imbalance between damaging and cytoprotective factors [3]. Damage factors can be endogenous (enhanced gastric acid and pepsin secretion, refluxed bile acids) or exogenous (alcohol, caffeine, tobacco, *Helicobacter pylori,* ingestion of non-steroidal anti-inflammatory drugs). Cytoprotective factors include an intact mucus barrier, prostaglandins, cellular regeneration, mucosal blood flow, activity of antioxidant compounds, epidermal growth factor, among others [4]. Nitric oxide is also implicated in regulation of gastric acid secretion and modulation of gastric mucosal integrity [5].

Ethanol-induced gastric ulcers have been widely used in the evaluation of gastroprotective activity due to its reproducibility,

Abbreviations: cox, cyclooxygenase; NSAIDs, non-steroidal anti-inflammatory drugs; GTN, goniothalamin; NEM, *N*-ethylmaleimide; L-NAME, *N*-ω-L-arginine methyl ester; ULI, Ulcer lesion index; ED_{50}, effective $dose_{50}$; NPSH, non-protein sulfhydryl compounds; NO, nitric oxide; NOS, nitric oxide synthase; PG, prostaglandin; ROS, reactive oxygen species; SH, sulfhydryl; DNTB, 5′5-dithiobis(2-nitrobenzoic acid); PPI, proton-pump inhibitors; Nrf2, nuclear factor erythroid 2 [NF-E2]-related factor 2; Keap 1, Kelch like-ECH-associated protein 1; ARE, antioxidant response element.

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fast action and the close relation between gastrointestinal diseases and alcohol consumption [6,7]. The genesis of ethanol-induced gastric lesions is multifactorial, involving decrease in gastric mucus and prostaglandin synthesis and increase in the production of free radicals, which in turn causes damage to cells [6].

Together with ethanol consumption, continuous use of non-steroidal anti-inflammatory drugs (NSAIDs) is also a major etiologic agent of gastrointestinal diseases. NSAIDs affect the gut by causing topical injury to the mucosa and systemic effects associated with mucosal prostaglandin depletion derived from cyclooxygenase-1 (COX-1). COX-1 isoform is expressed in most tissues and is responsible for production of prostaglandins, which play an important protective role in the gut by stimulating the synthesis and secretion of mucus and bicarbonate, increasing mucosal blood flow and promoting epithelial proliferation [8].

NSAIDs inhibit cyclooxygenases and thereby reduce the intrinsic resistance of mucosa against endogenous and exogenous aggressors. Indomethacin (IND), a representative of NSAIDs family, causes gastric ulcers through various processes, including generation of reactive oxygen species (ROS), initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis and inhibition of prostaglandin synthesis [9].

The most common drugs used in the treatment of gastric ulcers are antacids, anticholinergics, proton pump inhibitors (PPI) and histamine H_2-receptor antagonists. However, the large number of adverse effects caused by these therapies highlight the need for safer and more effective antiulcer agents [10]. In this search, bioactive natural products represent a privileged source of new potential therapies.

The styryl lactone goniothalamin (GTN, Fig. 1) is a secondary metabolite naturally found in its enantiomeric (*R*) form in plants of the genus *Goniothalamus* (Annonaceae) and presents *in vitro* antiproliferative effects in different human tumor cell lines [11–15].

Studies conducted by our group reported the synthesis of the non-natural isomer (*S*)-GTN and of several analogues obtained from goniothalamin, as well as their *in vitro* antiproliferative activity against a panel of human tumor cell lines [16–20]. Our group also reported the *in vivo* activity of (*R*) and (*S*)-GTN as well as of racemic form (*rac*-GTN) in both Ehrlich solid tumor and carrageenan-induced paw edema in mice, without side effects in the effective doses [21]. From these studies, the α,β-unsaturated δ-lactone moiety emerged as the key structural element which is proposed to act as a Michael acceptor for cysteine residues or other nucleophiles in biological systems [22].

Despite the abundant data on the *in vitro* goniothalamin biological activity, little is known about its *in vivo* pharmacological action. The present study describes the gastroprotective activity of racemic goniothalamin on ethanol- and indomethacin-induced gastric ulcers in rats, as well as the mechanisms of gastroprotection involved on ethanol-induced gastric lesions. Focus is given on the role of prostaglandins, nitric oxide, sulfhydryl compounds, mucus and gastric secretion.

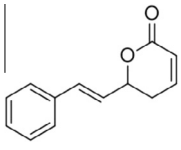

Fig. 1. Chemical structure of racemic goniothalamin.

2. Materials and methods

2.1. Synthesis of racemic goniothalamin

The racemic form of goniothalamin was synthesized in gram quantities according to previously described methodology from our group, except for the utilization of allylmagnesium bromide in substitution to the enantioselective allylation step. Starting from commercially available cinnamaldehyde, the Grignard reaction with commercially available allylmagnesium bromide provided the corresponding homoallylic alcohol in 90% yield, after column chromatography on silica gel. The homoallylic alcohol was converted to the corresponding acrylate with commercially available acryloyl chloride (80% yield), which was converted to racemic goniothalamin after ring closing metathesis reaction with 10 mol% of commercially available Grubbs type I catalyst (3 steps and 65% overall yield from cinnamaldehyde) [11,16]. Racemic goniothalamin was purified by flash chromatography on silica gel and its purity was confirmed to be >95% by ^{1}H- and ^{13}C-analyses and melting point comparison (m.p. = 82–84 °C; lit. 81–82 °C) [11].

2.2. Animals

Experiments were conducted with male adult Wistar/Uni rats (300–400 g, three-month old animals) from the Multidisciplinary Center for Biological Investigation on Laboratory Animals Sciences (CEMIB-UNICAMP). Animals were maintained at the Animal facilities of Pharmacology and Toxicology Division, CPQBA, University of Campinas (Campinas, Brazil), under controlled conditions (22 ± 3 °C for 12 h light/dark cycle, free access to food and water). All rats were fasted for 16 h prior to each experiment because treatments were administered orally. Euthanasia was performed by deeping anesthesia followed by cervical dislocation.

Animal care, research and animal sacrifice protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Institute of Biology/UNICAMP — Ethical Committee for Animal Research (number 3435-1). Acute toxicity of GTN was evaluated by our group on a previous study [21].

2.3. Drugs

Goniothalamin was emulsified with 1% Tween 80 (SIGMA®) and dissolved in phosphate buffered saline (PBS), pH 7.0. Vehicle was 1% Tween 80 in PBS, for all experiments. Ethanol (J.T. BAKER®), Alcian blue (REAGEN QUIMIBRAS®), sucrose (INLAB®), magnesium chloride (SYNTH®), carbenoxolone, cimetidine, *N*-ethylmaleimide (NEM), indomethacin, *N*-ω-L-arginine methyl ester (L-NAME), glutathione from SIGMA®. Ranitidine was from GLAXOSMITHKLINE®.

2.4. Experimental assays

2.4.1. Ethanol-induced gastric ulcers: determination of effective dose$_{50}$ (ED_{50})

To induce lesions in the gastric mucosa, absolute ethanol was used as an ulcerogenic agent, as described by Robert [23]. Animals were distributed into five groups ($n = 6$) and treated orally with vehicle (10 mL/kg), carbenoxolone (200 mg/kg) and GTN (15, 30 and 60 mg/kg). One hour later, animals received an oral dose of absolute ethanol (4 mL/kg), and after 1 h, rats were euthanized, the stomachs removed, opened along the greater curvature and washed. The ULI (ulcerative lesion index) score for each animal was calculated according with Table 1 [24]: