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Mechanisms involved in the gastroprotective activity of esculin on acute gastric lesions in mice

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ABSTRACT

This work describes the gastroprotective actions of esculin (6,7-dihydroxycoumarin-6-o-glucoside) against indomethacin- or ethanol-induced lesions and verifies the role of nitric oxide, ATP-dependent K^+ channels, prostaglandins, transient receptor potential vanilloid 1 and antioxidant effects in the gastroprotective mechanism of esculin in the ethanol-induced gastric lesion model. The intragastric administration of esculin at doses of 12.5, 25 and 50 mg/kg was able to protect the gastric mucosa against ethanol (0.2 mL/animal p.o.), and esculin at doses of 25 and 50 mg/kg protected against indomethacin-induced lesions (20 mg/kg p.o.). Administration of L-NAME (10 mg/kg i.p.), glibenclamide (10 mg/kg i.p.) or indomethacin (10 mg/kg p.o.), but not capsazepine (5 mg/kg p.o.), was able to reduce the gastroprotection promoted by esculin (25 mg/kg) on the ethanol-induced lesions. Measurements of nitrite, a NO metabolite, were increased in the group that was pretreated with esculin. In terms of antioxidant activity as a gastroprotective mechanism of esculin, the results show that pre-treatment with esculin decreased the amount of GSH, increased SOD activity, did not interfere with the CAT activity and decreased both the MPO activity and the MDA amount. In conclusion, pre-treatment with esculin confers significant gastroprotective and antioxidant activity and leads to a reduction in gastric injury; the mechanisms underlying these effects include stimulation of endogenous prostaglandins, nitric oxide synthesis, opening of K_{ATP} channels and reduction of free radicals or modulation of antioxidant enzyme systems.

1. Introduction

The gastric mucous membrane is continuously exposed to potentially harmful agents, such as HCl, pepsin, bile acids, food seasonings, bacterial products and drugs. These agents are involved in the pathogenesis of gastric injury by promoting an increase in the secretion of gastric acid and pepsin, decreasing gastric blood flow, suppressing the output of endogenous prostaglandins, inhibiting the cellular proliferation and growth of the mucous membrane and altering gastric motility [1].

In physiological conditions, there is a balance between the aggressive factors (HCl, pepsin, bile and pancreatic enzymes) and the gastroprotective factors (mucus-bicarbonate, blood flow, prostaglandins and glutathione). Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDS), smoking, alcohol, trauma, sepsis, shock, *Helicobacter pylori*, and stress have been shown to contribute to gastric ulcer formation [2].

Indomethacin-induced gastric lesions are characterized by significant oxidative injury, reduced mucosal blood flow and reduced secretion of mucus/bicarbonate, mainly due to inhibition of PG secretion [3]. Ethanol-induced gastric lesions occur mainly due to intense infiltration in the sub-mucosa that promotes formation of ROS, decreased mucus, depletion of sulfhydryl groups and decreased blood flow, resulting in damage to the gastric mucosa [4]. This model has fundamental importance for scientific research due to the fact that we can utilize it to evaluate possible mechanisms by which substances can act to promote gastroprotection. The generation of ROS and pro-oxidative events are involved in the etiology of gastric lesions in these two models. Several antioxidants exhibit gastroprotective activity, such as quercetin [5] and curcumin [6].

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Esculin (6,7-dihydroxycoumarin-6-o-glucoside) is a coumarinic derivative found in *Aesculus hippocastanum* L. (Horse-chestnut). Their seeds have long been used to treat inflammatory and vascular problems. In Brazilian folk medicine, the tea prepared from the crushed seeds is used to protect against kidney stones and stomach pain. Esculin is known to be a 5- and 12-lipoxygenase inhibitor and to inhibit the production of leukotrienes and 5-hydroxyeicosatetraenoic acid through the lipoxygenase pathway [7]. In 2007, Zhao et al. used the dopamine-induced cytotoxicity model in human neuroblastoma SH-SY5Y cells to demonstrate that esculin inhibited dopamine-induced caspase-3 cleavage and decreased cell death, overproduction of ROS, morphological changes of nuclei and damage to antioxidant enzymes [8]. Esculin scavenges hydroxyl radicals and inhibits lipid peroxidation in the rat liver [9] and displays anti-inflammatory activity in both zymosan- and carrageenan-induced paw edema in mice [10]. The gastroprotective effect of esculin was also observed in rats by Martin et al. [11] in cold-restraint stress and pylorus ligation-induced ulcer models; however, neither its effect on indomethacin-induced lesions nor the gastroprotective mechanisms involved were investigated in this study. Thus, this study was developed to better characterize the potential gastroprotective actions of esculin in mice and to investigate the possible mechanisms involved.

2. Materials and methods

2.1. Animals

Male *Swiss* mice (25–35 g) were used in this study. Animals were kept in a temperature-controlled room at 25 ± 2 °C with a 12-h light/dark cycle, with food and water *ad libitum*.

The study was approved by the Ethics Committee for Animal Research at the Federal University of Ceará in Brazil, and it was conducted in accordance with the National Institute of Health in Bethesda, USA.

2.2. Drugs

Capsazepine, capsaicin, cyproheptadine, diazoxide, esculin, glibenclamide, indomethacin, L-arginine, N^G-nitro-L-arginine methyl ester (L-NAME), sodium nitrite and Tween 80 were purchased from Sigma–Aldrich® (St. Louis, MO, USA), N-acetyl-L-cysteine (NAC) from União Química® (São Paulo, SP, Brazil), Ranitidine from GlaxoSmithKline® (Rio de Janeiro, RJ, Brazil) and Misoprostol from Pfizer® (São Paulo, SP, Brazil).

2.3. Study of the gastroprotective activity of esculin in the ethanol-induced gastric lesion model

The acute gastric lesions were induced by intragastric administration of absolute ethanol in accordance with a previously described method [3]. *Male Swiss* mice were randomly divided into five groups and fasted for 15 h before the experiment, but mice had free access to water. The ethanol groups were orally administered (0.2 mL/animal) 60 min after the treatment with esculin (12.5, 25 and 50 mg/kg, p.o – ESC12.5, ESC25 and ESC50, respectively). These doses were chosen based on results from previous studies by the same group with coumarin and umbelliferone (unpublished data) and from the study by Martin et al. [11]. The esculin was dissolved in 3% Tween 80 in distilled water. Control mice were similarly treated with Tween. Cyproheptadine, a non-selective antagonist of 5-HT and histamine receptors (10 mg/kg; p.o. – CYP10), was used as a reference drug. All experimental groups consisted of eight mice. These treatments were performed by gavage with a metal orogastric tube. Thirty minutes after the administration of ethanol, the mice were killed by cervical dislocation, and the stomach was removed and opened along the greater curvature for examination. The total and injured stomach areas (glandular face) were measured by the *Image J* computer program and expressed in terms of the percent (%) of lesioned gastric area.

2.4. Histopathological assessment

Histological evaluation was performed on the glandular face of the stomach. Tissue samples were preserved in 10% buffered formalin and processed for routine paraffin block preparation. Sections about 4 mm thick were cut and stained with hematoxylin and eosin. The mucosal injury evaluation was performed under light microscopy by an experienced histologist blinded to the treatment regimen. The histopathological changes were assessed according to the following criteria that were previously described by Laine and Weinstein [12]: (1) edema (score 0–4), (2) hemorrhagic damage (score 0–4), (3) inflammatory infiltration (score 0–3), and (4) epithelial cell loss (score 0–3).

2.5. Evaluation of the role of nitric oxide (NO), ATP-dependent K^+ channels (K_{ATP}), prostaglandins and transient receptor potential vanilloid 1 (TRPV1) in the gastroprotective effect of esculin on the ethanol-induced gastric lesion model

To study the possible mechanisms of action, the experiments were conducted using esculin (25 mg/kg; intermediate dose between the effective doses tested) and the following drugs: L-NAME, an inhibitor of NO-synthase activity (10 mg/kg, i.p.); glibenclamide, a blocker of K_{ATP} channels (10 mg/kg, i.p.); indomethacin (10 mg/kg, p.o.); and capsazepine (CZP), a TRPV-1 antagonist (5 mg/kg, i.p.).

L-NAME or glibenclamide were administered 15 min before administration of esculin (25 mg/kg, p.o.), L-arginine (600 mg/kg, p.o.) or diazoxide (3 mg/kg, p.o.). Indomethacin (10 mg/kg, p.o.) was administrated 2 h before administration of esculin (25 mg/kg, p.o.) or misoprostol (50 μg/kg, p.o.). Capsazepine (5 mg/kg, i.p.) was given 30 min before administration of esculin (25 mg/kg, p.o.) or capsaicin (CPS) (0.3 mg/kg, p.o.). After 1 h of oral administration was induced gastric injury with ethanol.

All animals received absolute ethanol (0.2 mL) for the lesion induction. Thirty minutes after the administration of ethanol, the mice were killed and the stomach was removed for examination as previously described.

The dose selections for these drugs were based on our pilot experiments and on literature findings [13–16].

2.6. Measurement of total nitrite levels

The amount of stable nitrite, the end product of NO metabolism, in the gastric mucosa was determined by a colorimetric assay as described by Green et al. [17]. Briefly, 100 μL of gastric mucosa homogenate was mixed with an equal volume of Griess reagent that consists of equal parts of 1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride (NEED), 5% H_3PO_4 and distilled water and incubated at room temperature for 10 min. The absorbance was read at 540 nm on a microplate reader (UVM-340, Asys Hitech, Netherlands). The amount of nitrite was calculated from a $NaNO_2$ standard curve.

2.7. Study of the gastroprotective action of esculin in the indomethacin-induced gastric lesion model

Male *Swiss* mice were randomly divided into four groups and treated orally with vehicle (controls), esculin (25 and 50 mg/kg p.o.) or ranitidine, a H2 histamine receptor antagonist (20 mg/kg; p.o.). After 60 min, gastric lesions were induced in all groups by