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Gastroprotective effects of sulforaphane and thymoquinone against acetylsalicylic acid–induced gastric ulcer in rats

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ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form
29 February 2016
Accepted 11 March 2016
Available online 24 March 2016

Keywords:
Gastric ulcer
Non-steroidal anti-inflammatory drug
Sulforaphane
Thymoquinone

ABSTRACT

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) commonly cause gastric ulcers (GUs). We investigated the effects of sulforaphane (SF) and thymoquinone (TQ) in rats with acetylsalicylic acid (ASA)–induced GUs.

Materials and methods: Thirty-five male Wistar–Albino rats were divided into five groups: control; ASA; ASA with vehicle; ASA + SF; and ASA + TQ. Compounds were administered by oral gavage before GU induction. GUs were induced by intragastric administration of ASA. Four hours after GU induction, rats were killed and stomachs excised. Total oxidant status, total antioxidant status, total thiol, nitric oxide, asymmetric dimethylarginine, tumor necrosis factor-alpha levels, superoxide dismutase activity, and glutathione peroxidase activity in tissue were measured. Messenger RNA expression of dimethylarginine dimethylaminohydrolases, heme oxygenase-1 (*HO-1*), nuclear factor erythroid 2–related factor 2, and nuclear factor kappa-light-chain-enhancer of activated B cells were analyzed. Renal tissues were evaluated by histopathologic and immunohistochemical means.

Results: SF and TQ reduced GU indices, apoptosis, total oxidant status, asymmetric dimethylarginine, and tumor necrosis factor-alpha levels, nuclear factor kappa-light-chain-enhancer of activated B cells, and inducible nitric oxide synthase expressions ($P < 0.001$, $P = 0.001$). Both examined compounds increased superoxide dismutase activity, glutathione peroxidase activity, total antioxidant status, total thiol, nitric oxide levels, endothelial nitric oxide synthase, dimethylarginine dimethylaminohydrolases, *HO-1*, nuclear factor erythroid 2–related factor 2, and *HO-1* expressions ($P < 0.001$).

Conclusions: These results suggest that pretreatment with SF or TQ can reduce ASA-induced GUs via anti-inflammatory, antioxidant, and antiapoptotic effects. These compounds may be useful therapeutic strategies to prevent the gastrointestinal adverse effects that limit nonsteroidal anti-inflammatory drugs use.

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0022-4804/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2016.03.027

Introduction

Gastric ulcers (GUs) are common gastrointestinal (GI) disorders that affect approximately 10% of the world population.[1] They are multifactorial and complicated disorders resulting from breakdown of the equilibrium between the defense mechanisms of the gastric mucosa and aggressive factors (e.g., secretion of gastric acids and pepsin; oxidants; free radicals; leukotrienes; endothelin) and exogenous factors (e.g., *Helicobacter pylori* infection, ethanol, nonsteroidal anti-inflammatory drugs [NSAIDs]). Conversely, prostaglandins (PGs), nitric oxide (NO), antioxidant molecules and enzymes (e.g., superoxide dismutase [SOD], glutathione peroxidase [GSH-Px]), gastric secretion of mucus and bicarbonate, and normal gastric blood flow help to defend the gastric mucosa.[2,3]

NSAIDs are used commonly worldwide but carry the risk of serious GI side effects that can result in erosions, hemorrhagic gastritis, and GUs. NSAIDs damage the stomach by inhibiting the cyclooxygenase (COX) enzyme system, thereby resulting in reduced levels of PGs. Moreover, NSAIDs impair gastric mucosal defensive mechanisms directly. Exposure of the gastric mucosa to NSAIDs leads to pathologic changes and the development of inflammation, hemorrhagic erosions, and GUs with generation of reactive oxygen species (ROS) and secretion of inflammatory cytokines.[4,5]

Sulforaphane ([1-isothiocyanate-(4R)-(methylsulfinyl) butane]; SF) is a dietary isothiocyanate found mainly in cruciferous vegetables such as broccoli and cabbage. SF has an indirect antioxidant capacity and can induce release of cytoprotective proteins and antioxidant enzymes such as heme oxygenase-1 (HO-1), glutathione reductase, and glutathione-S-transferase. It acts directly over nuclear factor erythroid 2–related factor-2 (*Nrf2*), which regulates the redox state and transcription of several antioxidant enzymes in various cell types. It has been reported that SF prevents oxidative damage in the brain, kidney, liver, heart, and pancreatic β-cells.[6,7]

Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone; TQ) is the main constituent obtained from *Nigella sativa*. TQ is an antioxidant and cytoprotective compound that inhibits lipid peroxidation. TQ is known to have several beneficial effects (hepatoprotective, antidiabetic, antibacterial) and to be involved in modulation of the immune system.[8,9]

Although there are many drugs used in GU treatment, all of them have side effects and they are not curative respectively.[10] Antioxidant and anti-inflammatory treatments may be promising approaches, and the harmful effects of ROS could be diminished by antioxidant drugs.

Animal models mimicking human disease have made considerable contributions to the clarification of the pathophysiological mechanisms of diseases and development of new strategies to treat and prevent such diseases. Acetylsalicylic acid (ASA)–induced gastric ulceration is an experimental model used widely to investigate the beneficial effects of such therapeutic strategies. In the acidic environment of gastric juices, high doses of ASA become deionized and penetrate the mucosal barrier readily. Thus, massive erosions and bleeding are elicited within minutes. Pathophysiological changes after ASA administration are comparable with those taking place in human GUs.[11,12]

In light of the potential protective effects of SF and TQ, clarification of the mechanisms of action of these compounds is important. In the present study, the mechanisms of action of SF and TQ in rats were examined through biochemical, molecular, histopathological, and immunohistochemical parameters.

Materials and methods

Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee for Experiments on Animals of Dumlupinar University (number 2014.09.01). This study was carried out in the Experimental Animals Research Center of Dumlupinar University. All experiments were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council.[13]

Animals

Male Wistar–Albino rats (300-350 g, 12-16 wk, $n = 35$) were used. Rats were housed individually in transparent polycarbonate cages with a 12-h light–dark cycle at 22 ± 2°C, and the animals were deprived of food for 24 h before the experiment but provided *ad libitum* access to fresh water.

Chemicals

DL-SF (PubChem CID: 10281) and ASA (PubChem CID: 2244) were purchased from Sigma-Aldrich Co LLC (Sigma-Aldrich Co LLC, St. Louis, MO). TQ (PubChem CID: 5350) and corn oil (CO) were purchased from Santa-Cruz Biotechnology (Santa-Cruz Biotechnology Inc, TX). ASA was dissolved in sterile, pyrogen-free distilled water, and SF and TQ were dissolved in 10% CO.

Experimental study design

Rats were divided randomly into five groups of seven. In group 1 (control), GU was not induced and no treatment given. In group 2 (GU), GU induction was undertaken using 1-mL ASA (150 mg/kg body weight, i.g.) and no treatment given.[12] In group 3 (vehicle), 1 h before GU induction, 1-mL CO (10%) was given by orogastric gavage. In group 4 (TQ + GU), 1 h before GU induction, TQ (20 mg/kg) was given by orogastric gavage.[14] In group 5 (SF + GU), 1 h before GU induction, SF (5 mg/kg) was given by orogastric gavage.[6]

Surgical procedures

Four hours after GU induction, rats were weighed and anesthetized with xylazine hydrochloride (10 mg/kg, i.m.; Rompun; Bayer, Munich, Germany) and ketamine (70 mg/kg, i.m.; Ketalar; Pfizer, New York, NY).[12] Then, the rats were stimulated for carpopedal reflex. After a suitable level of anesthesia was achieved, rats were placed on a homeothermic table to