Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Toxicology and Pharmacology 21 (2006) 86–92
ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY ETAP
www.elsevier.com/locate/etap

Antisecretory and antiulcer effects of diphenyl diselenide

Lucielli Savegnago, Marcio Trevisan, Diego Alves, Joao Batista Teixeira Rocha, Cristina W. Nogueira, Gilson Zeni *

Laboratorio de Sintese, Reatividade e Avaliacao Farmacologica e Toxicologica de Organocalcogenios, Centro de Ciencias Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

Received 20 December 2004; accepted 7 July 2005
Available online 6 September 2005

Abstract

The antisecretory and antiulcer effects of diphenyl diselenide were studied in vivo and in vitro. Diphenyl diselenide, administered intraperitoneally prevented the development of gastric lesions induced by ethanol and indomethacin. There was no difference in plasma uric acid concentrations in diphenyl diselenide-treated rats with gastric lesions induced by 70% ethanol. There were no changes in TBARS levels in diphenyl diselenide-treated rats with gastric lesions induced by indomethacin and ethanol. Diphenyl diselenide (5, 10 and 50 mg/kg) inhibited gastric acid secretion in pylorus-ligated rats. In vitro results demonstrated that diphenyl diselenide inhibited lipid peroxidation induced by Fe^{2+}/ascorbate/H_2O_2 and reduced K^+-dependent ATPase activity. The mechanisms by which pre-administered diselenide protects the damaged area in the gastric mucosa are not clear but it appears that the antiulcer activity of diphenyl diselenide is the result of antisecretory activity, via inhibition of gastric K^+-ATPase activity.

Keywords: Selenium; Organoselenium; Ebselen; Gastric lesion; Antisecretory

1. Introduction

It is widely accepted that the pathogenesis of the peptic ulcer is complex and still not completely understood. Increased acid secretion and pepsin activity, reduced mucus and bicarbonate secretion, enhanced contractility of the gastric wall and reduced gastric mucosa blood flow represent some of the established pathogenic factors of gastric ulceration (Galunska et al., 2002).

The mechanism which induces gastric lesions has been studied with various experimental models of ulcers by a number of researchers. Gastric ulcers induced by indomethacin are caused by the suppression of prostaglandin biosynthesis and the disruption of the gastric mucosal barrier (Ohe et al., 1980). Ethanol has been shown to produce gastric damage by impairing gastric defensive factors, such as mucus and mucosa circulation (Szabo et al., 1992). Moreover, ethanol and indomethacin-induced gastric damage may be caused by oxygen radicals and lipid peroxidation.

Reactive oxygen species (ROS) provoke severe changes at the cellular level leading to cell death because of their extreme reactivity. They attack essential cell constituents, such as proteins, lipids and nucleic acids, leading to the formation of toxic compounds (Kaharaman et al., 2003). The other fundamental factor in the pathogenesis of gastric and duodenal ulcers, besides ROS, is the presence of acid (Hunt, 1995). Therefore, the control of acid secretion may be essential for the treatment of these diseases. While acid secretion by parietal cells is regulated through several stimulatory receptors, such as histamine H_2, muscarinic M_3 and gastrin, the final step is mediated by gastric H^+, K^+-ATPase, the so-called proton pump (Hersey et al., 1995). Thus, the effective therapeutic control of acid secretion involves both the blockade of these receptors and the inhibition of the proton pump.

Gastric H^+, K^+-ATPase inhibitors have been considered effective remedies for acid-related diseases. Among the inhibitors, ebselen, an organoselenium compound, has been shown to inhibit parietal cell acid secretion through interference with sulfhydryl groups of the gastric proton pump, H^+, K^+-ATPase (Beil et al., 1990).

In addition, ebselen is known to possess glutathione (GSH) peroxidase-like activity, anti-inflammatory and antioxidant properties (Parnham and Graf, 1987). Accordingly, the employment of organoselenium compounds, as potential therapeutic antioxidant agents, has been indicated by many (Nogueira et

* Corresponding author. Tel.: +55 55 32208140; fax: +55 55 32208978.
E-mail address: gzeni@quimica.ufsm.br (G. Zeni).

1382-6689/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2005.07.017

al., 2003, 2004). Besides, ebselen has been used with success in human neuropathological situations (Saito et al., 1998) and in animal models of neurotoxicity (Porciúncula et al., 2001; Rossato et al., 2002).

The mechanisms that underlie the pharmacological potential of ebselen, are not completely understood. However, it has been postulated that the antioxidant properties of the drug might be, at least in part, responsible for its pharmacological action (Takasago et al., 1997).

The present study was designed to examine the pharmacological properties of diphenyl diselenide, as an antiulcer agent in gastric lesions induced by ethanol and indomethacin. We also investigated possible mechanisms responsible for the antiulcer effect of diphenyl diselenide.

2. Materials and methods

2.1. Animals

Male pigs (150–200 kg) were obtained from the Department of Zootechny, Pig Breeding Center, Federal University of Santa Maria, Santa Maria, RS, Brazil. Male adult Wistar rats (200–250 g) from our own breeding colony were used. The rats were kept in separate animal rooms, on a 12 h light/dark cycle, at room temperature, with free access to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of Sao Paulo, Brazil.

2.2. Materials

Ebselen and diphenyl diselenide were synthesized by the methods previously described (Engman, 1989). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that ebselen and diphenyl diselenide presented analytical and spectroscopic data in full agreement with their assigned structures. Organoselenium compounds were dissolved in dimethylsulfoxide (DMSO). All other reagents were of analytical grade and obtained from standard commercial suppliers. The commercial kit was obtained from LABTEST Diagnostica S.A., Minas Gerais, Brazil.

2.3. Ex vivo and in vivo experiments

2.3.1. Ethanol-induced gastric lesions

The gastric lesions were evaluated according to the method described by Robert (1979). The lesions were induced in 36 h fasted rats. The control group received the vehicle (DMSO, 1 mL/kg, i.p.) 1 h before the oral administration of 70% ethanol. Diphenyl diselenide was administered i.p. at different doses (0.001–50.0 mg/kg, 1 mL/kg) 1 h before the oral administration of 70% ethanol (v/v, 2 mL/kg, p.o.) (treated groups). The diphenyl diselenide group received an intraperitoneal injection of diphenyl diselenide and did not receive ethanol. This group did not appear in the results because diphenyl diselenide per se did not induce gastric lesions. The ethanol group received only the oral administration of 70% ethanol (v/v, 2 mL/kg, p.o.). This group did not appear in the results because there was no difference from the control group (DMSO + ethanol).

One hour later, each animal was killed under deep ether anesthesia. Subsequently, the stomachs were removed to determine the gastric lesion index and lipid peroxidation levels.

2.3.2. Indomethacin-induced gastric lesions

A modification of the method of Urushidani et al. (1977) was used. Gastric lesions were induced in 36 h fasted rats. Diphenyl diselenide was administered (i.p.) at different doses (0.01–50.0 mg/kg, 1 mL/kg) 1 h before the oral administration of 25 mg/kg indomethacin (1 mL/kg) in 5% sodium bicarbonate solution (treated groups).

Five hours later, the animals were killed under deep ether anesthesia. The stomachs were removed to determine the gastric lesion index and lipid peroxidation levels. Control groups were the same as described above (Section 2.3.1). The untreated group corresponds to the group treated with no drugs.

2.3.3. The gastric lesion index

The stomachs were removed, opened along the greater curvature and fixed in shapes to determine the gastric lesion index. The ulcerative lesion index of each animal was calculated by adding the following values (Gamberini et al., 1991), loss of normal morphology (one point), discoloration of mucosa (one point), mucosa edema (one point), ulcers up to 1 mm (one point), ulcers greater than 1 mm and less 2 mm (two points), ulcers greater than 2 mm and less 4 mm (three points) and ulcers greater than 4 mm (four points). The size of the gastric lesions was measured and compared to the control group. Lesions were homogenous and of the same extension in all animals of the same group. The observer of gastric lesions was blind to the treatments.

2.3.4. Uric acid

Blood was taken by cardiac puncture before the stomach was removed. Plasma was separated by centrifugation at 2500 rpm for 10 min and all manipulations were performed at 4–8 °C. Uric acid in the plasma and gastric mucosa homogenate was determined by KIT Labtest Diagnostica, S.A.

2.3.5. Lipid peroxidation

The content of gastric lipid peroxidation was determined using the method described by Ohkawa et al. (1979).

2.3.6. Antisecretory effect of diphenyl diselenide

The antisecretory effect of diphenyl diselenide was determined using pylorus-ligated rats according to Shay et al. (1945). Rats were starved for 24 h before experimentation. The pylorus of the rats was bound under thiophental (250 mg/kg) anesthesia. Diphenyl diselenide (5.0, 10.0 and 50 mg/kg) was administered to the rats by intraduodenal injection. The control group was given DMSO (1 mL/kg). Three hours after surgery, the stomach was isolated and the accumulated gastric juice was collected. After centrifugation of the gastric juice at 5000 rpm for 10 min, the supernatant was analyzed for gastric acid volume secreted per hour and pH. The acid was titrated with 0.1N NaOH using