MECHANISM OF PROTECTIVE EFFECTS OF DEHYDROLEUCODINE ON GASTROINTESTINAL TRACT. ROLE OF CAPSAICIN-SENSITIVE SENSORY NERVES

María AOM*, Wendel GH, Villegas C, Giordano O, Pelzer L

Farmacología. Química Orgánica. Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, San Luis, CP: 5700

INTRODUCTION
Infusions of fresh leaves of *Artemisia douglasiana* Besser (Asteraceae), popularly known as “mático”, are used in folk Argentinean medicine as a cytoprotective agent, to treat peptic ulcers, and as antispasmodic (1). Dehydroleucodine (DhL), a sesquiterpene lactone of the guaianolide type isolated from *Artemisia douglasiana*, shows a pharmacological cytoprotective effect and prevents the formation of gastric lesions induced by various necrotizing agents (2). DhL prevented the damage and inflammation in acetic acid-induced colitis in rats and TNBS-induced colitis in mice (3).

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active ingredient accounting for the pungency of hot peppers and has important pharmacological actions. Capsaicin is uniquely selective for stimulation and then blockade of the subset of mammalian afferent neurons of dorsal root ganglia with C and Aδ fibers. Sensory nerves have been shown to participate in the maintenance of the gastric mucosal homeostasis and in the protection against mucosal damage in the gastrointestinal tract (4).

In the present study, the role of capsaicin-sensitive neurons in the cytoprotection of DhL on gastric damage and experimental colitis was evaluated.

MATERIALS AND METHODS

**Extraction and purification of DhL.** *Artemisia douglasiana* Besser was collected in the mountains of the province of San Luis, Argentina, and a voucher specimen was deposited in the Herbarium of the Universidad Nacional of San Luis (UNSL No. 55). DhL was extracted as previously described (2). Briefly, the air-dried material was soaked in chloroform at room temperature. The extracts were evaporated in vacuo and dissolved in 95% ethanol. After addition of 4% aqueous lead tetraacetate solution, the aqueous cloudy solution was filtered through a celite pad, and the filtrate was concentrated under vacuum. The mixture was extracted 3 times with chloroform and the solution was concentrated under vacuum. The final residue was chromatographed in a medium-pressure chromatography system using 1:9 EtAcO/hexane as eluent. DhL (100% purity) was identified by 1H- or 13C-nuclear magnetic resonance, mass spectrometry, or melting point analysis, and its structure was identical to that cited by others authors (2).

**Animals:** Male Wistar rats, weighing 200-250 g were used. They were maintained in a restricted access room, which was temperature controlled at 26ºC. The light-dark cycle of the room was 12 h/12 h. They were provided with food and water *ad libitum*. All experiments were in compliance with the ANMAT No. 6344/96 for animal care guidelines.

**Functional ablation of afferent neurons:** Rats were treated subcutaneously with capsaicin in increasing doses (20, 30, and 50 mg/kg) on three consecutive days in a regimen shown to deplete neuropeptides in primary afferent neurons. The animals were pretreated with orciprenaline 0.2 mg/kg, atropine 0.2 mg/kg and theophylline 20 mg/kg; i.m., just before capsaicin injection to prevent the respiratory impairment associated with capsaicin injection (5). Experiments were performed two weeks after completion of the capsaicin treatment in animals fasted 24 h. One day before the start of treatment, functional ablation of the capsaicin-sensitive nerves was confirmed. One drop of 0.1 mg/ml capsaicin was instilled into one eye. Vehicle-pretreated rats responded with immediate wiping of the front paw against the eye instilled with capsaicin. The test eye was rinsed with water. The wiping response was absent in the capsaicin-pretreated rats.

**Production of acute gastric lesions:** Gastric lesions were produced according to the methods of Robert *et al.* (6). Absolute ethanol administered orally was employed as the necrotizing agent, and 1 h later the animals were sacrificed by CO₂ asphyxiation. DhL (100 mg/kg) was administered 1 h before the absolute ethanol. The stomachs were removed, opened along the greater curvature, and washed gently with ice-cold saline solution.

*Corresponding author (*). Tel +54 2652 424689, fax +54 2652 431301; e-mail: alemaria@unsl.edu.ar
The degree of erosion in the glandular part of stomach was assessed from a scoring system designed by Marazzi, Uberti and Turba (7).

**Production of experimental colitis:** Colitis was induced by 2 ml 10% acetic acid (i.r.). Colon rats received saline, acetic acid or capsaicin alone. Another group of rats received DhL 1 h prior to damage induction. Rats were sacrificed 24 h after damage induction, the colon isolated and damage was quantified by the scoring system of Wallace *et. al.* (8). Diarrhea was graded according to the criteria: 0 (normal) to 3 (severe) (9).

**RESULTS**

Administration of absolute ethanol produced severe band-like lesions with congestions in the corpus mucosa; the mean lesion index in the ethanol control group was 4.60 ± 0.24. DhL inhibited the formation of gastric lesions (0.10 ± 0.10, p<0.001 vs. ethanol control). In the control group of chemical ablation of capsaicin-sensitive sensory neurons and absolute ethanol, the lesion score was 4.50 ± 0.28. Sensory desaferentation abolished the protective effects of DhL on ethanol-induced gastric ulceration (0.85 ± 0.14, p<0.01 vs. DhL + ethanol).

All acetic acid-treated rats experienced diarrhea manifested as watery, loose stools. Acetic acid induced extensive colonic damage (8.2± 0.58). No damage was observed in the colon of rats treated only with saline or capsaicin. DhL pretreatment significantly decreased the macroscopic damage (1.21 ± 0.34, p<0.001 vs. acetic acid) and the diarrhea (p<0.001 vs. acetic acid). Capsaicin pretreatment resulted in significant reduction of the cytoprotective action of DhL (3.25 ± 0.45, p>0.05 vs. DhL + acetic acid).

**CONCLUSIONS**

In order to study the role of sensory nerves in the protection on gastrointestinal tract, we used capsaicin, an excito-toxin known to acutely stimulate unmyelinated (C) or thinly myelinated (Aδ) afferent neurons. With high doses or prolonged exposure to capsaicin, afferent neurons are functionally desensitized, exhibiting long-lasting loss of responsiveness to capsaicin itself or other stimuli of sensory neurons. The gastric mucosa is densely innervated by capsaicin-sensitive afferent neurons and these are known to increase the resistance of the gastric tissue to injury, facilitating the repair of damaged tissue by releasing peptide transmitters from their nerve endings. As a consequence, functional ablation of afferent nerves by a neurotoxic dose of capsaicin fails to induce acute injury by itself, but aggravates the mucosal lesions caused by topical irritants. Our results suggest that the protective activity of DhL on gastrointestinal tract is mediated, at least in part, through the afferent sensory neurons.

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**REFERENCES**