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Comparative study on the gastroprotective potential of some antidepressants in indomethacin-induced ulcer in rats

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ABSTRACT

Clinical studies have shown that anxiolytic and antidepressant drug therapy benefits patients with ulcers. Many antidepressant drugs have been shown experimentally to produce antiulcer activity in various ulcer models. This study investigated the antiulcer activities of tianeptine, trazodone, and venlafaxine on indomethacin-induced ulcers in rats; and evaluated tianeptine's effects on oxidant and antioxidant parameters in rat stomach tissue. The results show that trazodone and venlafaxine did not prevent indomethacin-induced ulcers. Tianeptine, however, decreased indomethacin-induced ulcers significantly at all doses used (6, 12, and 25 mg/kg). Famotidine, an H_2 receptor blocker, showed the highest antiulcer activity. Tianeptine significantly prevented the decrease in glutathione (GSH) content that occurred in the indomethacin-only group's damaged stomach tissues. All doses of tianeptine, but especially the 25 mg/kg dose, significantly decreased catalase (CAT) activity in stomach tissue, compared to the control. All doses of tianeptine eliminated the decrease in superoxide dismutase (SOD) activity in the stomach tissue of rats given indomethacin. Although all doses of tianeptine significantly decreased the malondialdehyde (MDA) content, all doses of tianeptine, except 6 mg/kg, decreased myeloperoxidase (MPO) activities significantly compared to the control. Our results indicate that activating enzymatic and non-enzymatic antioxidant mechanisms and inhibiting some toxic oxidant mechanisms play a role in tianeptine's antiulcer effect mechanism.

1. Introduction

A gastric ulcer is a multi-etiologic chronic disease. Various factors, such as the impairment of the balance between aggressive (increased acid secretions) and protective factors, stress, trauma, sepsis, hemorrhagic shock, burns, pulmonary and liver diseases, helicobacter pylori, use of cigarettes and alcohol, and steroidal and non-steroidal drugs, have been shown to play a role in gastric ulcerogenesis [1,2]. Apart from these factors, depression with psychotic and somatic symptoms has been seen in patients with gastrointestinal tract (GIT) diseases [3]. Clinical studies on this issue have shown that anxiolytic and antidepressant drug therapy benefits patients with ulcers [4]. A study performed by Hernandez et al. on rats showed that imipramine dose dependently prevents cold-stress-induced gastric lesions [5]. Also, another study has shown that imipramine and amitriptyline dose dependently prevent gastric ulcers in different ulcer models [6]. Disimipramine, an active metabolite of imipramine, has also potentially inhibited gastric acid secretion and produced gastroprotective effects in various ulcer models [7–9]. Many antidepressant drugs (e.g., fluoxetine, bupropion, dothiepin, maprotilin, mianserin, trimipramine, idozoxan, monoamino oxidase-B inhibitors, etc.) have been shown experimentally to elicit antiulcer activity in various ulcer models [10–14]. Researchers have shown that not only preclinical, but also clinical, studies suggest that antidepressant drugs exhibit antiulcer activities [15,16]. However, there is no information in the literature about the antiulcer effects of tianeptine, trazodone, or venlafaxine. For this reason, this study aims to investigate the antiulcer activity of tianeptine, trazodone, and venlafaxine on rats; and then to evaluate the effects of drugs whose antiulcer effect has been determined on oxidant and antioxidant parameters in rat stomach tissue.

2. Materials and methods

2.1. Animals

This study used 66 male albino Wistar rats weighing between 200 and 210 g, obtained from the Medical Experimental Research

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Centre, Ataturk University. The animals were fed under normal conditions (22 °C) in separate groups. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals, and approved by Ataturk University's local animal care committee.

2.2. Chemicals

All chemicals for laboratory experimentation were purchased from Sigma Chemical Co. Indomethacin, tianeptine, trazodone, and venlafaxine were obtained from Deva Holding-Istanbul, Servier-Istanbul, Çınay Chemistry Industry-Istanbul, and Wyeth Drugs-Istanbul, respectively.

2.3. Indomethacin-induced ulcer test

The antiulcer activities of tianeptine, trazodone, and venlafaxine were investigated on an indomethacin-induced ulcer model in rats [17]. For dose selection, we first determined a dose that had been used previously on rats; then we planned to study this effective dose, its half dose and its double dose. Tianeptine (6, 12, and 25 mg/kg doses) [18,19], trazodone (12, 25, and 50 mg/kg doses) [20,21], venlafaxine (12, 25, and 50 mg/kg doses) [22,23], and famotidine (20 mg/kg) were administered to rat groups by oral gavage. An equal volume of distilled water was administered to the control group as vehicle. Five minutes after the drugs' administration, all groups received 25 mg/kg indomethacin by oral gavage. Six hours after indomethacin's administration, all rat groups were killed with a high dose of thiopental sodium (50 mg/kg). The stomachs of all the rats were then excised. Ulcer areas on the stomachs' surface were examined macroscopically and measured on square millimeter paper. Any macroscopically visible lesions were measured to calculate the gastric damage score. For this purpose, the ulcerous stomachs were ingrained on a planar surface with small pins. Then the total areas of the stomachs and ulcerous areas were drawn on a cellophane sheet, which was put on millimeter paper. The sum of the ulcerous areas was expressed in mm^2 as the ulcer score. The drugs' antiulcer activities were evaluated by comparing the results obtained from the control and famotidine (20 mg/kg) groups with the following formula:

$$\begin{aligned}\text{antiulcer effect} &= \%\ \text{protection}\\ &= 1 - [\text{ulcer score of treatment group}/\\ &\quad \text{ulcer score of control group (indomethacin)}]\\ &\quad \times 100.\end{aligned}$$

2.4. Biochemical analyses

2.4.1. Biochemical investigation of stomach tissues

After the macroscopic analyses, the glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPO), and malondialdehyde (MDA) enzyme activities in rat stomach tissues were assessed. To prepare the tissue homogenates, the stomach tissues were ground with liquid nitrogen with a mortar. The ground tissues (0.5 g each) were then treated with 4.5 mL of appropriate buffer. The mixtures were homogenized on ice using an Ultra-Turrax homogenizer for 15 min. The homogenates were then filtered and centrifuged using a refrigerated centrifuge at 4 °C. Then these supernatants were used to determine the enzymes' activities. All assays were carried out at room temperature in triplicate.

2.4.2. Total glutathione (GSH) determination

The gastric mucosa's GSH content was measured according to the method of Sedlak and Lindsay [24]. The stomach's mucosal surface was collected by scraping, weighed, and homogenized in 2 mL of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 mM sucrose, at pH 7.5. The homogenate was immediately precipitated with 0.1 mL of 25% trichloroacetic acid, and the precipitate was removed after centrifugation at 4200 rpm for 40 min at 4 °C. The supernatant was used to determine GSH using 5,5'-dithiobis(2-nitrobenzoic acid). Absorbance was measured at 412 nm using a spectrophotometer. The results of the test for GSH content in the gastric mucosa were expressed as nanomoles per milligram of tissue (nmol/mg tissue).

2.4.3. Catalase (CAT) activity

Decomposition of H_2O_2 in the presence of CAT was followed at 240 nm [25]. CAT activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute, at 25 °C and pH 7.8. Results were expressed as millimoles per minute per milligram of tissue (mmol/min/mg tissue).

2.4.4. Superoxide dismutase (SOD) activity

Measurements were made according to Sun et al. [26]. SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitrotetrazolium blue (NTB) to form formazan dye. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction, and was expressed as millimoles per minute per milligram of tissue (mmol/min/mg tissue).

2.4.5. Myeloperoxidase (MPO) activity

MPO activity was measured according to the modified method of Bradley et al. [27]. The homogenized samples were frozen and thawed three times, and centrifuged at 1500 × *g* for 10 min at 4 °C. MPO activity in the supernatant was determined by adding 100 mL of the supernatant to 1.9 mL of 10 mmol/L phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L *o*-dianisidine hydrochloride containing 0.0005% (w/v) hydrogen peroxide. Each sample's changes in absorbance at 450 nm were recorded on a UV–vis spectrophotometer. MPO activity in gastric tissues was expressed as micromoles per minute per milligram of tissue (μmol/min/mg tissue).

2.4.6. Determination of lipid peroxidation or malondialdehyde (MDA) formation

The concentrations of gastric mucosal lipid peroxidation were determined by estimating malondialdehyde using the thiobarbituric acid test [28]. The rat stomachs were promptly excised and rinsed with cold saline. To minimize the possibility of hemoglobin's interference with free radicals, any blood adhering to the mucosa was carefully removed. The corpus mucosa was scraped, weighed, and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added to a solution containing 0.2 mL of 80 g/L sodium lauryl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL of distilled water. This mixture was heated at 98 °C for 1 h and after it had cooled, 5 mL of *n*-butanol:pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The supernatant's absorbance was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. The results were expressed as nanomoles MDA per gram of wet tissue (nmol/g tissue).

2.4.7. In vitro linoleic acid peroxidation assay

Tianeptine's antioxidative activity assayed by using the thiobarbituric acid (TBA) method based on the extract's inhibition of linoleic acid peroxidation. Linoleic acid was chosen as the source of