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Therapeutic effect of paroxetine on stress-induced gastric lesions in mice

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ABSTRACT

Compared to the well-known anti-ulcerogenic properties of tricyclic antidepressants, the impact of selective serotonin reuptake inhibitors (SSRIs) on gastric mucosa is less clear. Human clinical trials have shown that SSRIs and non-steroidal anti-inflammatory drugs (NSAIDs) act synergistically and promote stomach ulcer formation and upper gastrointestinal tract bleeding. Acute SSRI treatment confers an additional risk for the formation of NSAID-induced gastric ulcers through increase in gastric acid secretion. Stress, which is often experienced by depressed patients, also deteriorates the gastric environment. Thus the potential for exacerbating stress-induced gastric lesions must be considered before prescribing SSRIs. Therefore, we evaluated the effects of paroxetine by using a water-immersion stress-induced stomach ulcer model of mice, by examining single vs. repeated paroxetine treatments for 8 and 22 days before stress induction. Repeated administration of paroxetine significantly decreased the area of stress-induced stomach lesions. Although stress significantly increased the serum corticosterone concentrations, the levels were not affected by the 8-day paroxetine treatment. We confirmed the anxiolytic and antidepressive effects of 8-day paroxetine treatment at 1 and 5 days after stress induction by using the elevated plus-maze and tail-suspension tests. We concluded that repeated paroxetine treatment significantly attenuates the stress-induced ulcerogenic process in the stomach.

1. Introduction

Among antidepressant medications, the tricyclics are the best understood in terms of their effect on gastric ulcers. Through the anticholinergic and anti-histaminergic activities, they reduce gastric acid secretion and consequently exert an anti-ulcerogenic effect (Ries et al., 1984; Tsai and Yellin, 1984). Much less is known about the role of selective serotonin reuptake inhibitors (SSRIs) on the gastric mucosa. Stress and non-steroidal anti-inflammatory drug (NSAID)-induced lesions are the major experimental models for gastric ulcer formation in animals.

The effects of acute and single antidepressant pretreatment for gastric lesions have primarily been examined in an NSAID-induced stomach ulcer model of fasting male rats, with or without 1 h of refeeding just before NSAID administration. NSAIDs induce either antral lesions of the pylorus or broad stomach lesions of the gastric corpus, depending on whether refeeding is commenced before NSAID treatment (Satoh et al., 1982). Although a dose-dependent antiulcer effect of fluvoxamine on indomethacin-induced stomach ulcers has been recently reported (Dursun et al., 2009), an aggravating effect of paroxetine (PRX) on aspirin-induced gastric bleeding has been described (Yamaguchi et al., 2008). In these studies, inhibition of the cytochrome P450 enzyme, CYP1A2, by fluvoxamine appeared to reduce the production of toxic reactive oxygen species in the stomach, whereas increased gastric acid secretion due to co-administered PRX was presumed to elevate ulcerogenic risks. Increased gastric acid secretion by fluoxetine and sertraline via vagal nerve stimulation in rats has also been reported (Abdel Salam, 2004). The most consistent result from human studies shows that SSRI treatment alone has no serious detrimental effects on the gastric mucosa (Itatsu et al., 2011), but the concurrent use of most SSRIs with NSAIDs has been shown to have an additive negative effect on gastric injuries, including upper gastrointestinal bleeding and complicated peptic ulcer disease (Lewis et al., 2008).

Other than anti-inflammatory drug-induced peptic lesions, neurotoxic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced duodenal ulcers in rats have been reported to be partially prevented by repeated fluoxetine treatments (Keshavarzian et al., 1990). In a recent study, a 14-day fluoxetine treatment ameliorated colonic damage induced by acetic acid in rats (Guemei et al., 2008).

Abbreviations: DMSO, dimethylsulfoxide; NSAIDs, non-steroidal anti-inflammatory drugs; PRX, paroxetine; SSRIs, selective serotonin reuptake inhibitors.

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Only a few studies have evaluated the influence of acute or repeated SSRI pretreatment on stress-induced lesions, and the results have been controversial. Acute administration of fluoxetine reduces immobilization stress-induced stomach ulcerogenesis in rats (Gabry et al., 2002). Another study showed an ameliorating effect of a 12-day imipramine treatment, but not PRX, on forced swim stress-induced ulcers in rats (Tejani-Butt et al., 2003). For each of these antidepressant drugs, repeated administration is required to bring about the antidepressive and stress-resilient profiles.

In this study, we investigate the effects of single and repeated administrations of PRX on stress-induced gastric lesions in mice. The resilient effects of repeated PRX administration were examined by 2 different behavioral paradigms. Serum corticosterone levels were measured to evaluate the possible involvement of the hypothalamic–pituitary–adrenal axis in gastric lesion formation.

2. Methods

2.1. Experimental animals

Seven-week-old male C57BL6J mice (Clea, Tokyo, Japan) were used. They were shipped by the supplier 1 week before the experiments were initiated. The animals were treated in accordance with the NIH Methods and Welfare Considerations in Behavioral Research with Animals (http://www.nimh.nih.gov/researchFunding/animals.cmf), and all the experiments were conducted according to protocols approved by the local animal ethics committee of the National Defense Medical College. The mice were housed in groups (4 to 6 mice per cage) in climate-controlled rooms (24 °C, 55% humidity, and lighting provided from 0600 h to 2000 h), with free access to food and water.

2.2. Drug treatment and stress induction

2.2.1. PRX administration protocol

The following 3 protocols of drug pretreatment were used: (1) single PRX or saline administration; (2) 8-day PRX or saline administration; and (3) 22-day PRX or saline administration. PRX hydrochloride (20 mg/kg; Sigma, St. Louis, MO) in saline with 10% dimethylsulfoxide (DMSO), or control saline with 10% DMSO was administered intraperitoneally for the determined period until the stress experiment. We used a concentration of 20 mg/kg PRX, the maximum dose reported to induce behavioral effects on mice (Consoni et al., 2006; Cryan et al., 2004), to observe a possible gastric toxicity by PRX itself.

2.2.2. Experimental stress induction

Thirty minutes after the last PRX or saline injection, stress was induced in the mice. Animals were food deprived overnight and then each was placed into a syringe made of acryl (diameter, 30 mm; length, 95 mm) that was vertically immersed in a water bath at 23 °C for 3 h (Yoneda et al., 1983). The syringe had a 5-mm wide longitudinal slit located on both the surface and circular bottom for complete water immersion (WEB1318, Sanplatec, Osaka, Japan). Water was filled to 60 mm from the syringe bottom. The top of the syringe was composed of a hard metal net hemisphere to help ease respiration. Sham-stress control animals were similarly food deprived, removed from their home cages, and placed in new breeding cages for 3 h.

2.3. Sacrifice and tissue collection

Immediately after stress induction, a subset of mice was decapitated to examine the stomach lesions and serum corticosterone concentrations. For the 8-day pretreated group, the serum was separated from total blood, collected in microtubes (BD Microtainer tube; BD Diagnostics, Franklin Lakes, NJ), and immediately stored at −80 °C. Mice not used for serum collection were euthanized with a lethal dose of pentobarbital.

2.4. Measurement of serum corticosterone concentrations

Frozen serum samples were thawed on ice and analyzed by enzyme-based immunoassay (mouse corticosterone kit; IDS, Boldon, UK), and the results were measured with a microplate reader (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

2.5. Fixation, preparation, and image analysis of mouse stomachs

The stomachs were excised and fixed in 10 ml of 4% w/v paraformaldehyde in phosphate-buffered saline for 5 min. During fixation, an additional 0.25 ml of 4% paraformaldehyde was slowly injected into the stomach from the pyloric end. Total and damaged areas on the surface of the stomach were analyzed by the Image-J software (National Institutes of Health, Bethesda, MD) from scaled images. The area of the lesion was manually determined by evaluating lesions with or without bleeding on a 20× to 40× magnified images. Six mice were used for the 1-day saline/stress; 7 for the 1-day PRX/stress; 10 for the 8-day saline/sham stress; 16 for the 8-day saline/stress; 10 for the 8-day PRX/sham stress; 17 for the 1-day PRX/stress; 6 for the 22-day saline/stress; and 7 for the 22-day PRX/stress groups. For microscopic analysis, specimens were dehydrated and embedded in paraffin by using a tissue processor (Tissue-Tek VIP M1500; Sakura, Tokyo, Japan), and serial sections with a thickness of 4 μm were cut using a sliding microtome (Pteratome CMR440; Sakura) and mounted on 3-aminopropyltrimethoxysilane-coated slides (Superfrost S8443; Matsunami, Osaka, Japan). After the paraffin was removed, the samples were rinsed with water and stained with standard hematoxylin and eosin.

2.6. Elevated plus-maze and tail-suspension tests

After the stress induction in the 8-day saline and PRX-pretreated groups, mice destined for behavioral tests were replaced in their cages. The elevated plus-maze test (MSM-509S; Neuroscience Inc., Tokyo, Japan) was performed 1 day after stress induction. The mice were placed individually on the center square facing an open arm. During the 5-min test period, the time spent by the mice in both open and closed arms was automatically recorded and analyzed by a video-tracking software (CompACT VAS; Muromachi, Tokyo, Japan). The tail-suspension test was performed 5 days after stress induction. Each mouse was tested in a cubicle (400×300×385 mm) while it was suspended from a tail hanger with adhesive tape wrapped around the tail (2.5–3 cm from the tip), 30 cm above the floor. The trial was conducted for 6 min, and the duration of immobility was measured automatically using Image J-XX (O'Hara & Co. Ltd, Tokyo, Japan), which is a modified program based on the Image J. For the behavioral tests, a total of 27 mice were used for elevated-plus maze testing and 15 other mice were used for the tail suspension test.

2.7. Statistical analyses

All data are presented as the mean ± standard error. Data were statistically analyzed using a 2-way or 1-way ANOVA with Tukey's multiple comparison test and Student's *t*-test using Prism software (GraphPad Software, San Diego, CA). Differences were considered significant at the level of $p<0.05$.

3. Results

A significant induction of stomach lesions was observed after induction of water-immersion stress in these experiments. In the 8- and 22-day PRX pretreatment groups, the size of damaged areas significantly reduced. The damaged stomach area/total stomach area, expressed as a percentage, in the 8-day pretreatment groups was as follows: 0.3% ± 0.2% (8-day saline/sham stress group; total stomach area, 250 ± 8 mm^2; n = 10), 14.6% ± 2.4% (8-day saline/stress group; total stomach