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A protease inhibitor against acute stress-induced visceral hypersensitivity and paracellular permeability in rats

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ABSTRACT

In the present study, we investigated the effects of camostat mesilate (CM), a synthetic protease inhibitor, on visceral sensitivity and paracellular permeability induced by the acute restraint stress. We also explored the possible mechanisms underlying these effects. The acute restraint stress was induced by wrapping the fore shoulders, upper forelimbs and thoracic trunk of Sprague–Dawley rats for 2 h. Either CM (30, 100 and 300 mg/kg) or saline was intragastrically administrated to the rats 30 min before the acute restraint stress. Visceral perception was quantified as visceral motor response with an electromyography in a subset of rats. Paracellular permeability was determined in another subset of rats. We found that the visceral sensitivity and paracellular permeability were significantly reduced in the CM-treated rats. Moreover, the fecal protease activity was decreased in the CM-treated rats. The ZO-1 protein expression was markedly reduced by the stress treatment, but this decrease was suppressed by CM administration. Our data indicated that CM could efficiently inhibit visceral sensitivity and paracellular permeability induced by the acute restraint stress in rats. Therefore, CM might be an effective drug for the treatment of irritable bowel syndrome.

1. Introduction

Irritable bowel syndrome is one of the most commonly diagnosed gastrointestinal disorders, with a prevalence (estimated from population-based studies) of about 5–15%. This disease is characterized by abdominal pain or discomfort and alteration in bowel habits (Drossman and Dumitrascu, 2006). The etiology and pathogenesis are unknown; altered gastrointestinal motility and visceral hypersensitivity may play a role. Furthermore, some studies show that irritable bowel syndrome patients often have increased levels of anxiety or psychological distress, and stressful life events are associated with the onset or exacerbation of irritable bowel syndrome symptoms (Rey et al., 2009; Savas et al., 2008). An animal model for acute restraint stress has been established to mimic the changes in visceral sensitivity observed in irritable bowel syndrome patients. This phenomenon involves both central and peripheral release of corticotropin releasing factor and subsequent neuronal activation of spinal as well as supraspinal regions (Crane et al., 2005; Gue et al., 1997). In addition, the acute restraint stress in rat has effects on stimulating ion secretion and reducing barrier function (Saunders et al., 1994). Several studies show that intestinal permeability in irritable bowel syndrome patients is increased (Piche et al., 2009; Quigley, 2009; Zhou et al., 2009). These observations suggest that rats treated with acute restraint stress can be used as a model of irritable bowel syndrome.

Recent advances have shown that in addition to their digestive role in protein degradation, proteases play a role as signaling molecules regulating cell functions by cleaving protease-activated receptors (PARs) (Amadesi and Bunnett, 2004). PARs are activated by a variety of proteases, such as digestive enzymes (trypsin and trypsinogen), proteases released from mast cells and neutrophils, proteases of the coagulation cascade and proteases from bacteria (Vergnolle, 2005). PARs activations may alter visceral nociception, mucosal permeability and intestinal motility, and it may also increase ion transport (Roka et al., 2008; Vergnolle, 2005). Recent studies also suggest that irritable bowel syndrome patients with diarrhea are characterized by an increased fecal serine-protease activity, and such elevated protease activity may contribute to an increased colonic paracellular permeability and subsequent visceral hypersensitivity, which are mediated by protease activated receptor 2 (Gecse et al., 2008). Colonic biopsies from irritable bowel syndrome patients also release increased proteolytic activity that may trigger visceral hyperalgesia in mice (Cenac et al., 2007).Other study reports that the trypsinogen IV expression at the mRNA level is enhanced in small intestinal mucosal biopsy specimens of irritable bowel syndrome patients compared with that of healthy subjects (Kerckhoffs et al., 2008). Therefore, we hypothesized that protease inhibitors might have a therapeutic potential for irritable bowel syndrome. To our knowledge, there has been no study on the therapeutic use of protease inhibitors in irritable bowel syndrome. In this study, we aimed to evaluate whether a protease inhibitor inhibited this colonic hypersensitivity and altered the paracellular permeability induced by acute restraint stress.

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2. Materials and methods

2.1. Animals

Eighty female Sprague–Dawley rats (180–220 g) were involved in this study with free access to water and food throughout the experiments. The rats were maintained in a controlled environment at 21±2 °C and 50±5% relative humidity under a 12-h light/dark cycle, and they were acclimatized for at least 1 week prior to experiment. The cycle of females was not assessed during the experiment. All experimental protocols described in this study were approved by the local committee for animal use and care (Animal Care Committee of Xi'an Jiaotong University).

2.2. Drugs

Camostat mesilate (CM) was purchased from Shengruixiang Pharmaceutical Company (Jinan, Shandong, China), and it was dissolved in distilled water and kept at room temperature. Monoclonal anti-β-actin antibody was purchased from Sigma Chemical Company (St Louis, MO, USA). Polyclonal anti-ZO-1 antibody and horseradish-peroxidase-conjugated goat anti-rabbit IgG secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Super Signal Substrate Chemiluminescence Kit was purchased from Pierce (Rockford, IL, USA).

2.3. Animal preparation

To measure rat's visceral sensitivity, the visceral motor response to colonic distention was quantified by the electromyography as previously described (Satio-Nakaya et al., 2008). Briefly, the rats were anaesthetized with 10% chloral hydrate (3 ml/kg) by intraperitoneal injection, and a pair of Teflon coated stainless wires (M1.5I; Star Medical, Shanghai, China) were implanted into the left external abdominal oblique muscle for electromyography recording. Wires were subcutaneously passed and exteriorized through the back of the neck. After the surgery, the animals were individually housed and allowed to recover for a minimum of 7 days before being used in experiments.

2.4. Acute restraint stress

Acute restraint stress procedure was operated as previously described (Gue et al., 1997). In order to minimize the influence of circadian rhythms, all stress sessions were performed during the same period of the day (between 10:00 AM and 12:00 noon). The animals were lightly anesthetized with ethyl-ether, and their fore shoulders, upper forelimbs and thoracic trunk were wrapped in a confining harness of paper tape to restrict, but not to prevent body movements. Subsequently, the rats were placed back to their home cages for 2 h. Rats recovered from ethyl-ether within 2–3 min and immediately moved around in their cages, although the restricted mobility of their forelimb prevented the grooming behavior. The sham-stress rats, considered as controls, were anesthetized as above but were not wrapped and were allowed to move freely in their cages. Rectal distension procedure started 30 min after the end of the stress period.

2.5. Rectal distension

The rats were placed into plastic tunnels (6 cm of diameter, 25 cm of length), where they could not move, escape or turn around. In order to minimize the stress reactions during experiments, the rats were accustomed to this procedure for 3 days before the rectal distension. A urethral catheter (8F, B. Braun Med Ind., Malaysia) was lubricated with liquid paraffin oil and intrarectally inserted 6 cm from the anus of rats under short ether anesthesia. The catheter was fixed to the rat tail with adhesive tape. Graded rectal distension stimulation (0, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) was applied in an ascending manner with 5 min intervals. Each distension lasted for 20 s, and it was done in triplicate to obtain an accurate estimate.

Electromyography was continuously recorded during the experiment with Power Lab System (AD Instruments International, Australia). Electromyography signals were amplified 5000 times, and then they were filtered with a 1 Hz high-pass filter and a 500 Hz low-pass filter. Electromyography activity was analyzed according to the changes of the area under the curve during the 20 s distention period, which was obtained by subtracting the 20 s baseline of each rat.

2.6. Experimental protocol

The rats were divided into 10 groups (eight rats per group). All animals were intragastrically administrated with either saline (groups 1, 2, 6 and 7) or protease inhibitor (groups 3, 4, 5, 8, 9 and 10) 30 min before the acute restraint stress (see Table 1). The animals fasted for 16 h before the acute restraint stress treatment. Groups 1 and 6 were used as the normal control and given the sham-stress. The rats in groups 2–5 and 7–10 were submitted to the acute restraint stress. In these two series, groups 6–10 intracolonically received 250 μl of ^{51}Cr-ethylene diamine tetra acetic acid (^{51}Cr-EDTA) (Perkin–Elmer Life Sciences, Paris, France) for 20 min after the stress or sham-stress, and the colonic paracellular permeability was assessed by 24 h post-stress urine collection. Groups 1–5 were submitted to a progressive rectal distension, which was performed 30 min after the stress or sham-stress.

2.7. Measurement of fecal protease activities

According to the study (Gecse et al., 2008), 30 min after the acute restraint stress, feces were collected and stored at −80 °C. Briefly, 1 g fecal sample was thawed, dissolved and homogenized in 7 ml of Tris buffer, and then it was centrifuged (4500 g, 10 min, 4 °C) and filtered (0.22 μm, Nalgene, San Diego, CA, USA). The supernatant was used to measure protease activity. Supernatants of fecal homogenates (25 μl) were incubated with 1 ml of reaction buffer (0.15 mol/l NaCl and 20 mmol/l Tris–HCl, pH 8.3) and 1 ml of 0.5% (w/v) azocasein (Sigma, St Quentin Fallavier, France) at 40 °C for 20 min. The reaction was terminated with 1 ml of 10% (v/v) trichloroacetic acid. Following the centrifugation (4500 g, 10 min, 4 °C), absorbance of the clear supernatant was measured at 366 nm. The protein concentration of the sample was assessed with BCA-200 Protein Assay Kit (Pierce, Rockford, IL, USA) with bovine serum albumin as a standard. Protease activity was expressed as units per milligram of protein, standardized against activity elicited by 1 U of standard trypsin.

2.8. Colonic paracellular permeability

Evaluation of colonic paracellular permeability was performed with ^{51}Cr-EDTA as a marker of paracellular permeation of tight junction (Ferrier et al., 2003). Experiments were carried out 20 min after the

Table 1
Description of the experimental protocol. All rats were divided into 10 groups. Groups 1–5 were used to measure the visceral sensitivity. Groups 6–10 were used to evaluate the paracellular permeability.

Groups	Animal model	Treatments
Groups 1,6	Sham-stress	Saline
Groups 2,7	Acute restraint stress	Saline
Groups 3,8	Acute restraint stress	30 mg/kg protease inhibitor
Groups 4,9	Acute restraint stress	100 mg/kg protease inhibitor
Groups 5,10	Acute restraint stress	300 mg/kg protease inhibitor