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Effectiveness of trimebutine maleate on modulating intestinal hypercontractility in a mouse model of postinfectious irritable bowel syndrome

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ABSTRACT

Trimebutine maleate, which modulates the calcium and potassium channels, relieves abdominal pain in patients with irritable bowel syndrome. However, its effect on postinfectious irritable bowel syndrome is not clarified. The aim of this study was to investigate the effectiveness of trimebutine maleate on modulating colonic hypercontractility in a mouse model of postinfectious irritable bowel syndrome. Mice infected up to 8 weeks with *T. spiralis* underwent abdominal withdrawal reflex to colorectal distention to evaluate the visceral sensitivity at different time points. Tissues were examined for histopathology scores. Colonic longitudinal muscle strips were prepared in the organ bath under basal condition or to be stimulated by acetylcholine and potassium chloride, and consecutive concentrations of trimebutine maleate were added to the bath to record the strip responses. Significant inflammation was observed in the intestines of the mice infected 2 weeks, and it resolved in 8 weeks after infection. Visceral hyperalgesia and colonic muscle hypercontractility emerged after infection, and trimebutine maleate could effectively reduce the colonic hyperreactivity. Hypercontractility of the colonic muscle stimulated by acetylcholine and high K^+ could be inhibited by trimebutine maleate in solution with Ca^{2+}, but not in Ca^{2+} free solution. Compared with 8-week postinfectious irritable bowel syndrome group, 2-week acute infected strips were much more sensitive to the stimulators and the drug trimebutine maleate. Trimebutine maleate was effective in reducing the colonic muscle hypercontractility of postinfectious irritable bowel syndrome mice. The findings may provide evidence for trimebutine maleate to treat postinfectious irritable bowel syndrome patients effectively.

1. Introduction

Irritable bowel syndrome is the most common, multifactorial disorder in gastroenterology practices, which affects about 12% of the population worldwide (Halvorson et al., 2006; Mertz, 2003). In recent years, acute gastroenteritis has been recognized to play a critical role in the development of irritable bowel syndrome (Neal et al., 1997; Parry and Forgacs, 2005; Rodríguez and Ruigómez, 1999). Studies showed that about one third of patients with irritable bowel syndrome described that their symptoms began after an acute enteric infection (Gwee et al., 1999; McKendrick and Read, 1994; Neal et al., 2002), and this kind of patients was recognized as a new subtype of postinfectious irritable bowel syndrome (Gwee et al., 2003; Spiller, 2003, 2007; Spiller et al., 2000).

Treatment of irritable bowel syndrome remains unsatisfactory, and cognitive behavioral treatment, psychotherapy, and hypnosis could provide long-lasting benefit in some patients (Lackner et al., 2006; Statements, 2009; Talley and Spiller, 2002). Drugs are almost symptomatic treatment, like antidiarrhoeals, bulking agents, 5-HT_3 antagonists, 5-HT_4 agonist, tricyclic antidepressants, and so on (Ford et al., 2009; Quartero et al., 2005; Tack et al., 2006). However, the evidence for the efficacy, safety and tolerability of such drugs was limited. The prognosis for patients with postinfectious irritable bowel syndrome is somewhat better than for those with unselected irritable bowel syndrome, but to date, no definitive therapy has been found (Neal et al., 2002; Thabane et al., 2007). Several studies showed that probiotics appeared to be efficacious in postinfectious irritable bowel syndrome, but the exact effects and target type of patients remain to be clarified (Moayyedi et al., 2008; Nikfar et al., 2008; Spiller, 2007).

Visceral hypersensitivity has been emphasized in postinfectious irritable bowel syndrome patients, which strongly responded abdominal pain to mild stimulation. Some antispasmodics such as trimebutine and pinaverium are believed to relieve pain associated with irritable bowel syndrome through inhibiting the contraction of muscle wall by modulating the muscle ion channels. Trimebutine, unlike the calcium-channel blocker pinaverium, has a more complicated function on both the calcium and potassium channels, which can improve the symptoms effectively by its dual modulating effects.

Trimebutine maleate has been efficiently used for the treatment of irritable bowel syndrome to relieve abdominal pain and alter bowel habits (Delvaux and Wingate, 1997; Poynard et al., 2001). In isolated

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intestines from guinea-pigs and rabbits, trimebutine maleate could stimulate or inhibit spontaneous contractions depending on the prior contractile activity in preparation (Takenaga and Tamaki, 1986). Trimebutine maleate had been reported to be active against rectal hyperalgesia induced by local inflammation and stress in rats (Lacheze et al., 1998). The effects of trimebutine maleate on gastrointestinal tracts were mediated by a Ca^{2+} antagonist-like action with inhibiting the influx of extracellular Ca^{2+} in the smooth muscles (Nagasaki et al., 1991; Shimada et al., 1990). Furthermore, trimebutine maleate also had an inhibit effect on the K^+ current evoked upon membrane depolarization of the gastrointestinal smooth muscles at the resting conditions to induce contractions (Nagasaki et al., 1993).

However, no research has been done towards the effects of trimebutine maleate to postinfectious irritable bowel syndrome. As for the ion channel modulating mechanism of this drug, as well as existing dysmotility and visceral hypersensitivity in the postinfectious irritable bowel syndrome patients, we determined to investigate whether trimebutine maleate could be used to treat postinfectious irritable bowel syndrome by modulating the hyperreactivity of the postinfectious intestines. A widely used mouse model of postinfectious irritable bowel syndrome using NIH Swiss mice infected with *T. spiralis* was studied here, which had shown sensory and motor changes after enteric inflammation subsidence (Barbara et al., 1997; Berck et al., 2004; Spiller, 2003; Sukhdeo and Croll, 1981).

2. Materials and methods

2.1. Mice and T. spiralis infection

The experimental procedure was approved by the Ethics Committee of Animal Experimentation, Tongji Medical College. Specific pathogen-free Male NIH Swiss mice, 6–8 weeks old, with free access to food (Standard pellets, Tongji Medical College, Wuhan, China) and water, were kept at a constant temperature (22–23 °C) and with lighting cycle of 12 h light/12 h dark. Animals were acclimatized to their environment for at least 7 days prior to the experiments. Thirty mice were randomly divided into three groups: control, acute infection group (2 weeks after infection), and chronic infection group (8 weeks after infection). Mice were infected by intragastric inoculation of 350–400 *T. spiralis* larvae in 0.1 ml phosphate-buffered saline (PBS). Sham-infected animals received 0.1 ml PBS. The larvae were obtained from male Sprague–Dawley rats infected 60–90 days previously by *T. spriralis* using a modified technique described by Castro and Fairbairn (Castro and Fairbairn, 1969). Mice were killed by cervical dislocation to obtain tissues for inflammation grading and colonic muscle strip studies. Intestinal samples were taken from ileocecum and the whole colonic samples from the proximal to distal colon. Sham-infected mice were killed at matched time points and data were collected for a single control group.

2.2. Experimental protocols

Thirty Male NIH Swiss mice were randomly divided into three groups: control, acute infection group (2 weeks after infection), and chronic infection group (8 weeks after infection). To evaluate the model, the following indexes were observed: body weights, inflammation histopathology, visceral sensitivity, and intestinal muscle contraction. Body weights of the mice were recorded every week after infection. Inflammation histopathology was assessed by HE staining of the ileocecum. Visceral sensitivity was evaluated by measuring behavioral responses of abdominal withdrawal reflex to colorectal distention. Intestinal muscle contractility was studied by measuring the contraction of the longitudinal muscle strips in the organ bath. To investigate the effectiveness of trimebutine maleate on the muscle strips, consecutive concentrations of trimebutine maleate were added into the bath with or without acetylcholine, K^+, or Ca^{2+} in it.

2.3. Abdominal withdrawal reflex recording to colorectal distention

After a 24 h fasting but ad libitum access to water, visceral sensitivity was measured by behavioral responses to colorectal distention, assessed by measuring abdominal withdrawal reflex using a semiquantitative score or by measuring the threshold intensity of colorectal distention that elicits an express contraction in the abdominal wall musculature (Al-Chaer et al., 2000). On the day of testing, mice were briefly sedated with halothane for balloon (an inflexible plastic balloon: length, 1.5 cm; diameter, 0.9 cm) insertion with liquid paraffin oil (Sigma, USA), and the balloon was inserted into the descending colon 2 cm from the anal verge, and secured by taping the attached tubing to the mouse tail. Then mice were placed inside a restraint device. They were allowed to recover 30 min fully from the halothane anesthesia and throughout the duration of colorectal distention testing. Colorectal distention was performed as described previously (Jones et al., 2007). Abdominal withdrawal reflex and thresholds were recorded during phasic balloon inflation to 15, 30, 45, 60, and 80 mm Hg by blinded observers. Abdominal withdrawal reflex score scale (Al-Chaer et al., 2000): 0, no behavioral responses to colorectal distention; 1, brief head movement followed by immobility; 2, contraction of abdominal muscles; 3, lifting of abdomen; 4, body arching and lifting of pelvic structures. Measuring the threshold intensity of colorectal distention consisted of recording the stimulus intensity that evokes a visually identifiable contraction of the abdominal wall. During the measurements, mice were given colorectal distention for 20 s every 4 min. To achieve an accurate measure, each pressure was repeated 5 times. The score at each pressure was presented as the means of the 5 times. Abdominal withdrawal reflex was measured at 2 and 8 weeks after infection, as well as before infection. The results obtained were compared among groups to determine the visceral sensitivity.

2.4. Histopathological study

Following the behavioral experiments, the ileocecum and colon were removed, placed in 10% formalin and processed for histopathology. HE staining was performed by standard techniques. The severity of the lesions in the intestine was graded as follows with a scale previously described by Al-Chaer et al. (2000). 1+, mild, infiltration of a limited number of neutrophils in the lamina propria with minimal or no interstitial edema; 2+, moderate, infiltration of a moderate numbers of neutrophils in the lamina propria and moderate interstitial edema; 3+, severe, diffuse infiltration of moderate to large numbers of neutrophils in the lamina propria with severe interstitial edema. There were 2 investigators to grade the inflammation blindly, and a mean total score was obtained.

2.5. Tissue preparation

Tissues were prepared for contractility studies as previously described (Barbara et al., 1997). The entire length of the colon was removed and dissected free of mesenteric attachments, gently cleaned of luminal contents with Krebs solution, and the mucosal layers were removed using forceps and scissors under binocular microscopic control. The 10 mm long segments of the colon were excised parallel to the longitudinal muscle by gently peeling off the muscle in longitudinal direction. They were placed in longitudinal direction in warmed (37 °C) 25 ml organ baths containing oxygenated ($95\%O_2/5\%CO_2$) Krebs solution. One end of the tissue was connected to an isometric force transducer (Fort-10, WPI, USA), and the other to the armature of the bath.

2.6. Contractility studies

Tissues were preloaded with 1.0 g, and allowed to equilibrate in the baths for 45 min until a stable baseline was attained. At the first stage of the experiment, increasing concentrations of trimebutine