DOCTORAL THESIS

Analgesic effect and the underlying mechanisms of JCM-16021 in TNBS-induced PI-IBS rats

Qin, Hongyan

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QIN Hongyan

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Principal Supervisor: Prof. BIAN Zhaoxiang

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ABSTRACT

Post infectious irritable bowel syndrome (PI-IBS) is a subset of IBS which occurs after an episode of acute gastrointestinal infections. Currently, the commonly used animal models of PI-IBS are induced either by gut parasite/bacteria infection, or by chemical agents. Up to now, PI-IBS sufferers are not satisfied with the current managements, because these agents primarily aim at symptomatic relief. JCM-16021, a revised classic Chinese herbal formula, has been proved to relieve symptoms in IBS patients and attenuate visceral hypersensitivity in a rat model of IBS, but the underlying mechanisms have not been well understood. The aims of this study are: i) to review the currently used animal models of PI-IBS; ii) to investigate the key factors in developing trinitrobenzene sulfonic acid (TNBS)-induced rat model of PI-IBS; iii) to investigate the analgesic effect and the underlying mechanism of JCM-16021 in a rat model of PI-IBS; and iv) to investigate the analgesic effect and the underlying mechanism of quercetin, one of the flavonoid in JCM-16021, in PI-IBS rats and an enterochromaffin (EC) cell model, QGP-1 cell line.

A systematic review was conducted to evaluate the existing animal models of PI-IBS by searching in Ovid SP database. Results showed that the currently used PI-IBS animal models could be categorized into post-infectious models and post-inflammatory models; TNBS was the most commonly used agent for post-inflammatory IBS model development, but its development protocol had large variations. In order to investigate the effects of TNBS dosage and concentration, intracolonic administration position, and ethanol percentage on developing the TNBS-induced rat model of PI-IBS, and to provide a standard procedure, the severity of colonic inflammation, visceral sensation, and serotonin hyperactivity-related indexes were assessed. We found that i) TNBS

induced dose- and concentration-dependent inflammation; ii) TNBS administration position affected the persistence of visceral hyperalgesia; iii) ethanol percentage affected the TNBS-induced inflammation severity and acquired visceral hyperalgesia; iv) TNBS (5 mg/0.8 ml/rat, in 50% ethanol, 8 cm from anus)-treated rats recovered completely with acquired visceral hyperalgesia and EC cell hyperplasia. These results indicate that TNBS dosage and concentration, position of TNBS administration, and ethanol percentage indeed play important roles in developing TNBS-induced PI-IBS rat model, and more attention should be paid to these factors when developing the rat model of PI-IBS.

In this study, the analgesic effect was evaluated by using abdominal withdrawal reflex (AWR) test and electromyographic (EMG) recording. We found that JCM-16021 treatment significantly and dose-dependently attenuated visceral hyperalgesia in the rat model of PI-IBS, indicating the analgesic effect of JCM-16021 in PI-IBS rats. Further, JCM-16021 treatment dose-dependently reduced colonic EC cell number, tryptophan hydroxylase (TPH) expression, 5-HT content and colorectal distention-induced 5-HT release in PI-IBS rats. These data suggest that the analgesic effect of JCM-16021 in the rat model of PI-IBS may be mediated through reducing colonic EC cell hyperplasia and 5-HT availability. In addition, JCM-16021 treatment significantly elevated and restored the reduced cytokines, especially the T helper 1 (T_h1) related cytokines, in PI-IBS model rats. These data suggest that JCM-16021 can modulate mucosal cytokines production in PI-IBS rats, and this modulation may contribute to the reduction of colonic EC cell number and 5-HT availability.

The analgesic effect of quercetin, a compound of Chinese herbal formula JCM16021,

and its underlying mechanisms were investigated using PI-IBS rats and QGP-1 cell line. The results showed that i) quercetin dose-dependently attenuated visceral hyperalgesia in the rat model of PI-IBS; ii) low and medium doses of quercertin significantly reduced colonic EC cell hyperplasia in the rat model of PI-IBS; iii) quercetin significantly decreased QGP-1 cell viability by inhibiting cells proliferation and inducing cytotoxicity; and iv) quercetin induced cell apoptosis and G_2/M phase cell cycle arrest in QGP-1 cells. These results indicate that the analgesic effect of quercetin may be mediated via reducing colonic EC cell hyperplasia and 5-HT availability, and the underlying mechanism may have correlation with its effect on inhibiting cell proliferation and inducing apoptosis. Further, these data may partially explain the analgesic effect of JCM-16021.

In summary, there are large variations among the procedure for developing PI-IBS animal model with TNBS. The optimized procedure for developing TNBS-induced rat model of PI-IBS is as follows: TNBS at 5 mg/0.8 ml/rat, dissolved in 50% ethanol, intracolonically administer at 8 cm from the anus. The analgesic effect of JCM-16021 in PI-IBS rats provides further supporting evidences for the therapeutic effect of JCM-16021 in IBS, also gives a clue that the reduced colonic EC cell number and 5-HT availability may contribute to the analgesic effect of JCM-16021. Moreover, the analgesic effect and mechanism of quercetin in PI-IBS rat model may enrich our understanding on the bioactivities of quercetin.

TABLE OF CONTENTS