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Research Article
Matrine Protects the Intestinal Barrier from Dysfunction via SPRY4-IT1 Signaling Pathway

[1]Shihua Chen, [2]Zihua Wang, [2]Bin Pi, [1]Chao Li, [1]Zhen Song, [1]Min Liu and [1,3]Zhihua Liu

[1]Department of Anorectal Surgery, The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510799, China
[2]Department of Emergency, The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510799, China
[3] Innovation Centre for Advanced Interdisciplinary Medicine, Key Laboratory of Biological Targeting Diagnosis,
Therapy and Rehabilitation of Guangdong Higher Education Institutes, The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Abstract

Background and Objective: Intestinal barrier dysfunction is associated with many clinical diseases. However, no efficient biomarkers and therapeutic drugs of intestinal barrier dysfunction have been found till now. In this study, the positive effects of matrine on intestinal barrier via SPRY4-IT1 was investigated. **Materials and Methods:** Expression of tight junction associated proteins was determined through SPRY4-IT1 inhibition or overexpression Caco-2 cells. DSS-induced mice colitis was used to verify the protective role of matrine on intestinal barrier and the regulation on SPRY4-IT1. SPRY4-IT1 and related was detected in clinical specimens of acute stercoral obstruction patients. **Results:** QRT-PCR showed that SPRY4-IT1 inhibitor could prohibit the role of matrine on intestinal barrier protection. The intestinal permeability of DSS-induced mice colitis was increased and the tight junction-associated proteins were decreased, which could be relieved by matrine. There was also a lower of SPRY4-IT1 in clinical colonic samples in stercoral obstruction patients. **Conclusion:** Matrine could protect the intestinal barrier from dysfunction via the signaling path way of SPRY4-IT1.

Key words: Intestinal barrier, matrine, occluding, SPRY4-IT1, tight junction

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Corresponding Author: Zhihua Liu, Department of Anorectal Surgery, The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510799, China Tel: +862082281638/Fax: +86 20 82297393

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As a widely-used traditional medicine, matrine gets a lot of therapeutic functions, such as antivirus, anti-oxide, anti-inflammation and anti-cancer[1]. It is reported with a structure of a naturally occurring alkaloid of herbs[2]. Till now, only a few studies reported the anti-colitis effects of matrine on mice models. The intestinal protective effects of matrine on mice colitis induced by IL-10-deficient mice[3] and 2,4,6-trinitrobenzene sulfonic acid[4,5] have been clarified. One study found that matrine could protect against DSS induced mice colitis by improving intestinal barrier structure, integrity and tight junctions, through the PPAR-α signaling pathway and gut microbiota[2].

Recently, more and more studies have investigated the role of noncoding RNAs[6,7]. Long non-coding RNAs (lncRNAs) are transcribed RNAs spanning more than 200 nucleotides in length without protein-coding potential[8]. The lncRNAs may regulate their target enes at different levels. It is reported that some lncRNAs may remodel chromatin, recruit transcriptional activators or suppressors, inhibit RNA-binding proteins (RBPs) and regulate protein assembly[8]. In contrast, others can alter the stability and translation of mRNAs and regulate microRNAs. Therefore, lncRNAs could regulate different biological processes[9,10], such as development, differentiation, apoptosis and proliferation Farh *et al.*[11] and Cao *et al.*[12]. Furthermore, lncRNAs are reported to be associated with intestinal barrier function as well[13,14]. SPRY4-IT1 was first identified as a 706–base pair transcript[15], present in sequencing of adipose tissue DNA. It was reported to be broadly expressed in human intestinal mucosa, which was derived from the intronic region of the SPRY4 gene[8]. Another study about SPRY4-IT1 found its protecting effects on the intestinal barrier and TJ[8]. It is also proven that patients with increased gut permeability showed a decreased SPRY4-IT1 expression level[8].

Our previous study has indicated the process and molecular mechanisms of TJ maintenance and protection[16-18]. However, the possible molecular mechanism and molecular signaling pathway of matrine during the preventive process of intestinal barrier dysfunction has not been fully elucidated. In addition, the relationship between matrine and SPRY4-IT1 has not been reported as the molecular mechanism of intestinal barrier protection. In this study, the molecular mechanisms of matrine in the protection of the intestinal epithelial barrier through the pathway of SPRY4-IT1 was investigated.

MATERIALS AND METHODS

The study was carried out in the central laboratory of The Fifth Affiliated Hospital of Guanzhou Medical University (The Guangzhou Key Laboratory of Enhanced Recovery Abdominal Surgery Laboratory) from 6th, 2018-2020.

Cell culture and reagents: Cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Life Technologies) media at 37 in a humidified atmosphere containing 5% CO_2, which was supplemented with 10% FBS, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Cell passage was performed using 0.05% trypin and 0.5 mm EDTA (Gibco, USA)[19]. Matrine was purchased for cell and animal tests from the Chengdu must bio-technology Co., Ltd. Lentivirus vectors or inhibitors were constructed by Shanghai Minzai Co., Ltd. The Caco-2 cells were transfected with Lipofectamine 2000 (Invitrogen, USA).

DSS-induced mice colitis: C57BL/6 mice for more than eight generations were bought from Guangdong Laboratory Animal Center (Guangzhou, China) and kept in Guangzhou Medical University Animal Center. As described by Liu *et al.*[20], mice colitis was induced using the DSS (MP, Biomedicals) method by adding 3.0% DSS in the drinking water for about a week. The blood was collected from their eyeballs, then the C57BL/6 mice were sacrificed to prepare the colonic specimens. Mice were given 20 mg kg^{-1} matrine via oral gavage once a day for pretreatment[2].

Permeability assay: Transepithelial electrical resistance (TER) and dextran permeability was used to evaluate the permeability of Caco-2 cells and DSS induced mice colitis[19]. The colonic permeability was evaluated by the fractional excretion for sucralose, while the small intestinal permeability was detected by the ratio of lactulose/mannitol of fractional excretion. The permeability of isolated specimens of mice colons could also be determined by using chamber assay as reported[19].

Quantitative Real-Time Polymerase Chain Reaction (QRT-PCR) analysis: Total RNA was extracted from cells or tissue samples using Trizol lysis buffer (Ambion, USA) according to the manufacturer's instructions. The RNA isolation of cell or tissue or serum samples was performed with the miRNeasy MiniKit (Qiagen, Germany) according to the manufacturer's instructions. The Reverse Transcription kit (Invitrogen, USA) was used to synthesize the first strand of cDNA. A SYBR MasterMix (Invitrogen, USA) and a 7,500 Real-Time

Table 1: PCR primer

Genes	Primers
IL-6	(F)5′-CCAGAAGACCAGAGGAAA-3′
	(R)5′-GGAAATCGTGGAAATGAG-3′
TNF-α	(F)5′-GACGTGGAACTGGCAGAAGAG-3′
	(R)5′-TTGGTGGTTTGTGAGTGTGAG-3′
Occludin	(F)5′-TTGAAAGTCCACCTCCTTACAGA-3′
	(R)5′-CCGGATAAAAAGAGTACGCTGG-3′
Zo-1	(F)5′-ACCAGTAAGTCGTCCTGATCC-3′
	(R)5′-TCGGCCAAATCTTCTCACTCC-3′

PCR system could help with qRT-PCR form RNA. The qRT-PCR form RNA was performed according to the manufacturer's instructions 102[21] (Table1).

Western blot analysis: Western blotting was performed by a wet electroblotter (Bio-Rad) for 120 min at 100 V as previous reported[17,22]. The enhanced chemiluminescence method (ECLkit, Pierce, Illinois) was selected for determination as described by Liu *et al*.[18].

Determination of clinical samples: Serum and tissue samples of acute stercoral obstruction patients were used to evaluate the expression level of SPRY4-IT1. Fresh colon and serum were collected from the stercoral obstruction patients. Colon and the paired adjacent normal colon tissues were used to determine the expression levels of SPRY4-IT1 and related inflammatory factors. Tissues were collected from patients in The Fifth Affiliated Hospital of Guangzhou Medical University, which was approved by the Scientific and Ethical Committee of The Fifth Affiliated Hospital of Guangzhou Medical University in accordance with the approved human subject guidelines.

Statistic analysis: Statistical data were analyzed by GraphPad Prism 5 software (San Diego, CA) and expressed as mean±SEM and analyzed with Student's T-test. The $p<0.05$ was defined as significant.

RESULTS

Matrine could alleviate the decrease of the expression level of occluding induced by SPRY4-IT1 inhibitor in Caco-2cells: To determine the protective effects of matrine on occludin, the Caco-2 cell line was transfected with SPRY4-IT1 inhibitor. Results indicated that the expression of SPRY4-IT1 was significantly decreased after the transfection of SPRY4-IT1 inhibitor in Caco-2 cells ($p<0.05$, Fig.1a). Furthermore, although LPS might inhibit the expression of TJ associated protein occluding in Caco-2 cells, after the pretreatment of matrine, occludin expression was promoted and enhanced, while SPRY4-IT1 inhibitor deprived the protective effects of matrine ($p<0.05$, Fig. 1b).

Matrine could lower the expression of the signal of P38 MAPK, which could be Inhibited by SPRY4-IT1 inhibitor: LPS could higher the expression of P38MAPK in Caco-2 cells, significantly and matrine inhibited the effects of LPS, however, transfection of SPRY4-IT1 inhibitor lead to rose of the P38MAPK once more ($p<0.05$, Fig. 2a). Meanwhile, after SPRY4-IT1 precursor was transfected into Caco-2 cells, expression level of P38. The MAPK signaling decreased again, no matter with or without the pretreatment of matrine ($p<0.05$, Fig. 2b).

Treatment of DSS-induced mice colitis confirmed the relationship between SPRY4-IT1 and the signal molecular P38MAPK: TER level was significantly lower after treatment of matrine in Caco-2 cells ($p<0.05$, Fig. 3a), while the relative intensity was significantly higher, however, transfection of SPRY4-IT1 inhibitor could relieve the effects of matrine ($p<0.05$, Fig. 3b). Using chamber assay indicated a higher intestinal permeability of DSS-induced mice colitis compared with control, while the effects could be relieved by matrine pretreatment ($p<0.05$, Fig. 3c, d). The lactulose/mannitol rate and sucralose excretion rates could be used to measure the permeability of small intestine and colon, respectively. The lactulose/mannitol rate was increased in the DSS induced mice colitis and inhibited by matrine as well ($p<0.05$, Fig. 3e). Detection of sucralose excretion indicated same results ($p<0.05$, Fig. 3f).

The average body weight of DSS-induced mice was lower, compared with control, which could be relieved by matrine ($p<0.05$, Fig. 4a). Furthermore, serum IL-6, TNF-α and zonulin levels increased in the DSS-induced mice by the detection of PCR, compared to WT group significantly, which were relieved by matrine ($p<0.05$, Fig. 4b-d). Determination of occludin and ZO-1 in colon tissues also indicated a significant decrease for DSS-induced mice colitis, while the effects could be relieved by the pretreatment of matrine ($p<0.05$, Fig. 4e, f). The expression level of SPRY4-IT1 was also evaluated, which indicated that matrine could enhance the expression of SPRY4-IT1 in the DSS induced mice ($p<0.05$, Fig. 4g).

Low level of SPRY4-IT1 in clinical samples of acute stercoral patients: The level of SPRY4-IT1 in clinical colon tissues of stercoral obstruction patients. Significantly decreased than

control of the adjacent normal colon tissues ($p<0.05$, Fig. 5a). The occluding and ZO-1 expression levels from colonic tissues also declined significantly, compared with control ($p<0.05$, Fig. 5b, c). In contrast, expression of serum zonulin, IL-6 and TNF-α were increased in patients with acute stercoral obstruction ($p<0.05$, Fig. 5d-f).

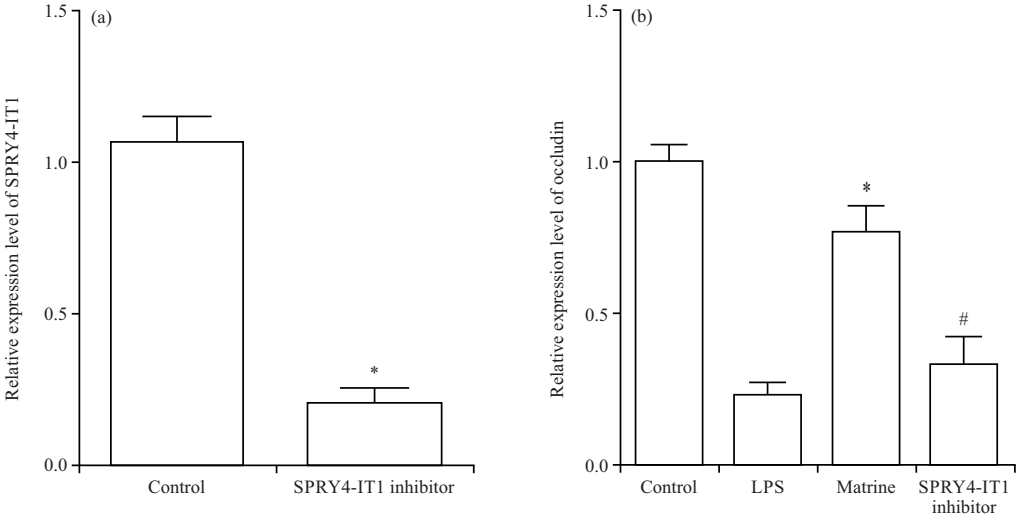

Fig. 1(a,b): Matrine could alleviate decrease of occluding expression induced by SPRY4-IT1 inhibitor, (a) Analysis of the relative expression level of SPRY4-IT1 determined by qRT-PCR in Caco-2 cells transfected with SPRY4-IT1 inhibitor and control and (b) LPS could lower the expression of occludin in Caco-2 cells. However, after the pretreatment of matrine, expression of occluding was increased, while SPRY4-IT1 inhibitor deprived the effects of matrine

*vs LPS group, $p<0.05$, *vs$^{\#}$ and $p<0.05$

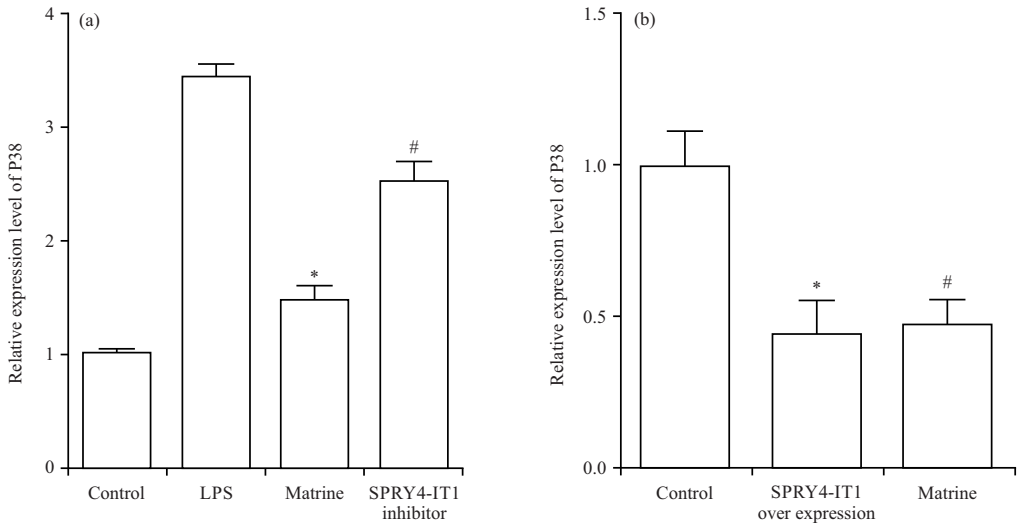

Fig. 2(a-b): Matrine downregulated the P38MAPK signaling, which could be inhibited by SPRY4-IT1 inhibitor, (a) LPS could higher the expression of P38 MAPK in Caco-2 cells, significantly and matrine could inhibit the effects of LPS, however, after SPRY4-IT1 inhibitor was transfected, P38 MAPK expression rose again and (b) Analysis of the relative expression level of P38 MAPK at mRNA level in SPRY4-IT1 precursor transfected Caco-2 cells and control with or without the pretreatment of matrine

*vs LPS group, $p<0.05$, *vs$^{\#}$ and $p<0.05$

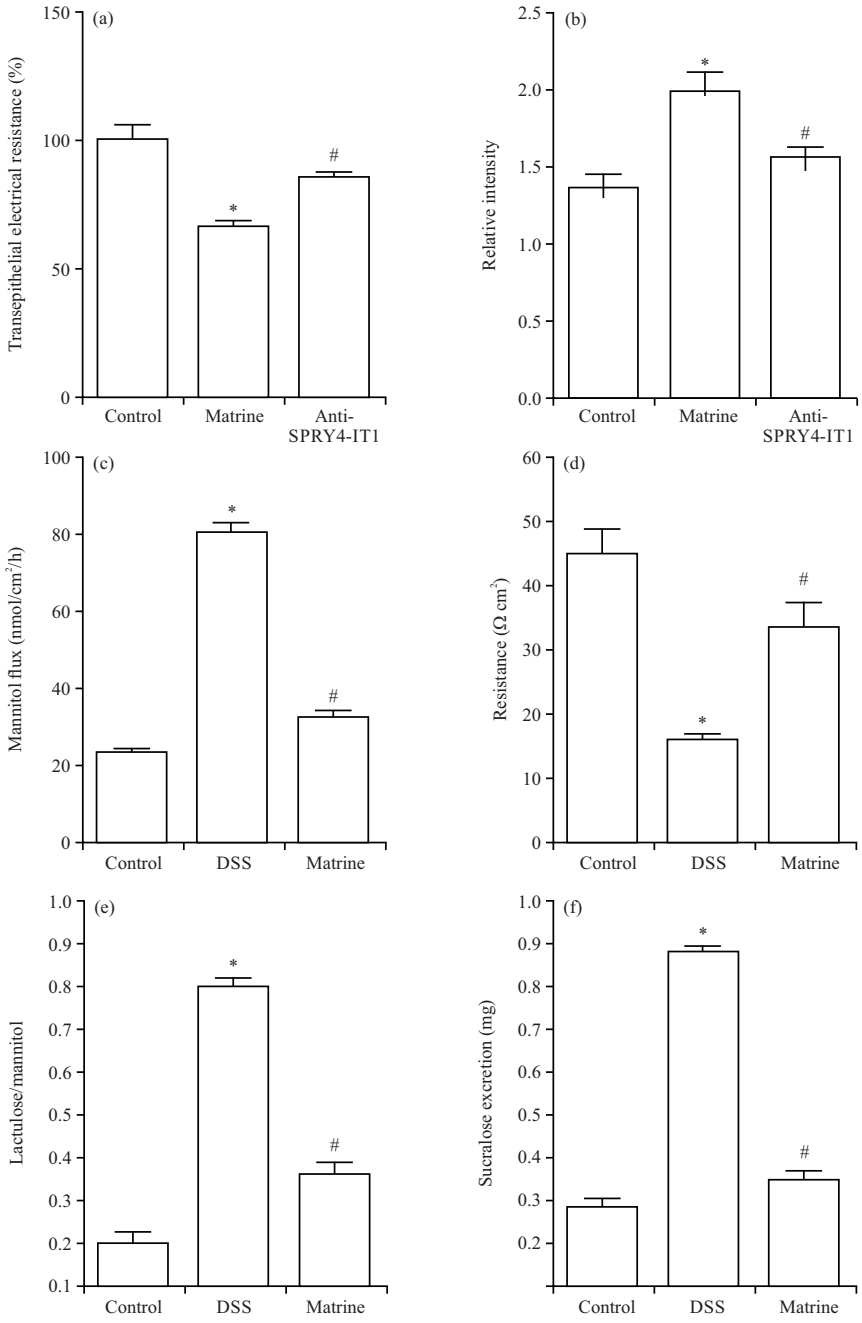

Fig. 3(a-f): Matrine could lower intestinal permeability through the inhibition of SPRY4-IT1 and ROCK-1 pathway, (a) Transepithelial electrical resistance was lowered after the pretreatment of matrine in Caco-2 cells, which could be relieved by the transfection of SPRY4-IT1 inhibitor, (b) The relative intensity was enhanced after the pretreatment of matrine, which could be relieved by transfection of SPRY4-IT1 inhibitor, (c) Mannitol flux was increased in the DSS induced mice colitis group, compared with the control wild-type group, which could be relieved by matrine, (d) Resistance was decreased in the DSS induced mice colitis group, compared with the control wild-type group, which could be relieved by matrine (e) Lactulose/mannitol rate was higher in the DSS induced mice colitis, compared with the control WT group at 8 and 12 week mice, which could be relieved by matrine and (f) Sucralose excretion was higher in the DSS induced mice colitis, compared with the control WT group at 8 and 12 week mice *in vivo*, which could be relieved by matrine

*p<0.05, *vs#, p<0.05 and Three independent experiments were performed for cell test and fifteen were performed for mice

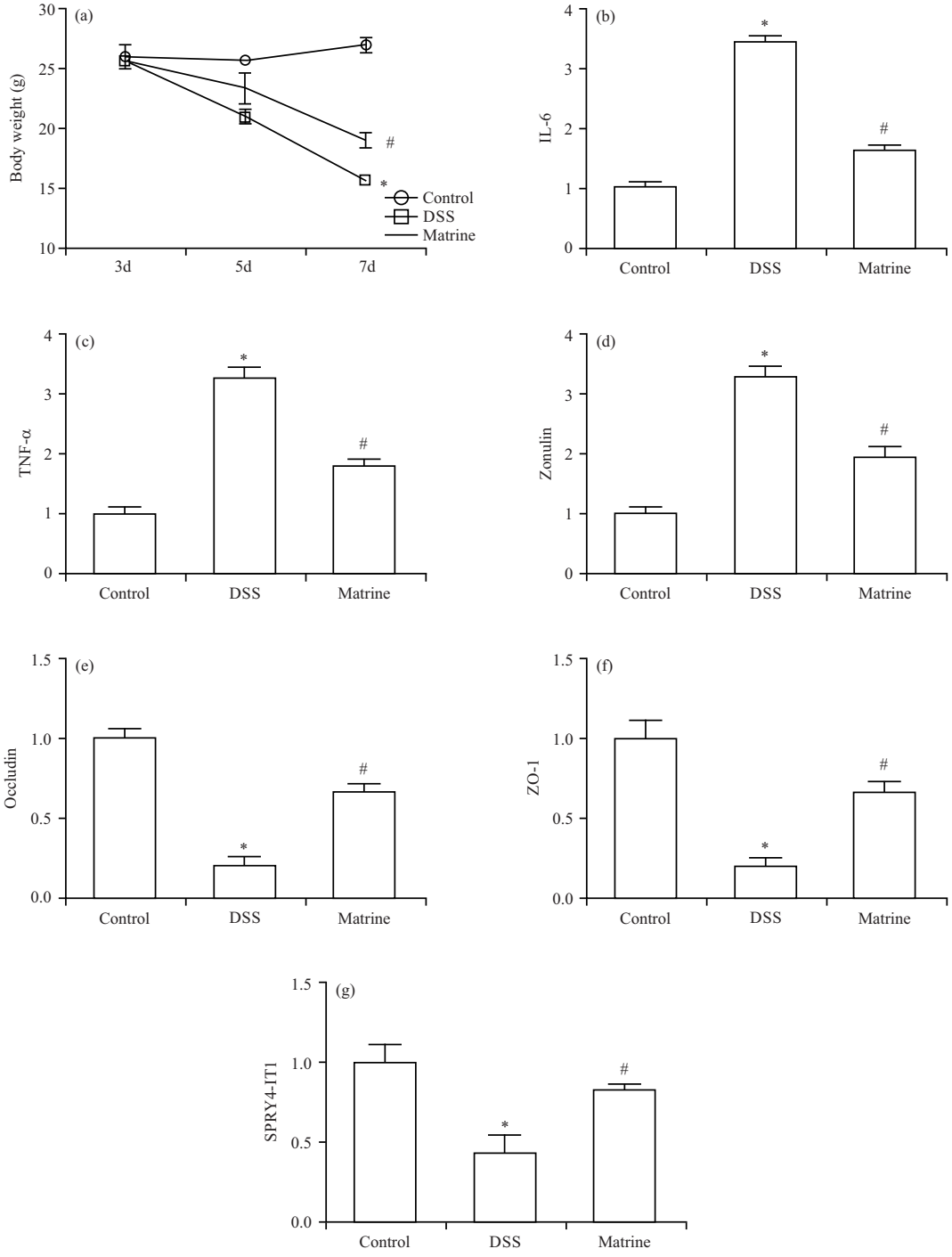

Fig. 4(a-g): Matrine could relieve the destruction of tight junction, (a) Body weight of DSS induced mice was lower, while after the pretreatment of matrine, the body weight became higher compared with the DSS group, (b-d) Serum levels of zonulin, IL-6 and TNF-α levels were higher in the DSS induced mice colitis group, compared with the WT group, which could be relieved by anti-ROCK-1, (e, f) Occludin and ZO-1 of colon tissues decreased in the DSS-induced mice colitis group, while the effects could be relieved by giving the drugs of matrine to mice and (g) Matrine could enhance the expression of SPRY4-IT1 in DSS induced mice colitis model

*p<0.05, *vs#, p<0.05 and Fifteen independent experiments were performed for mice

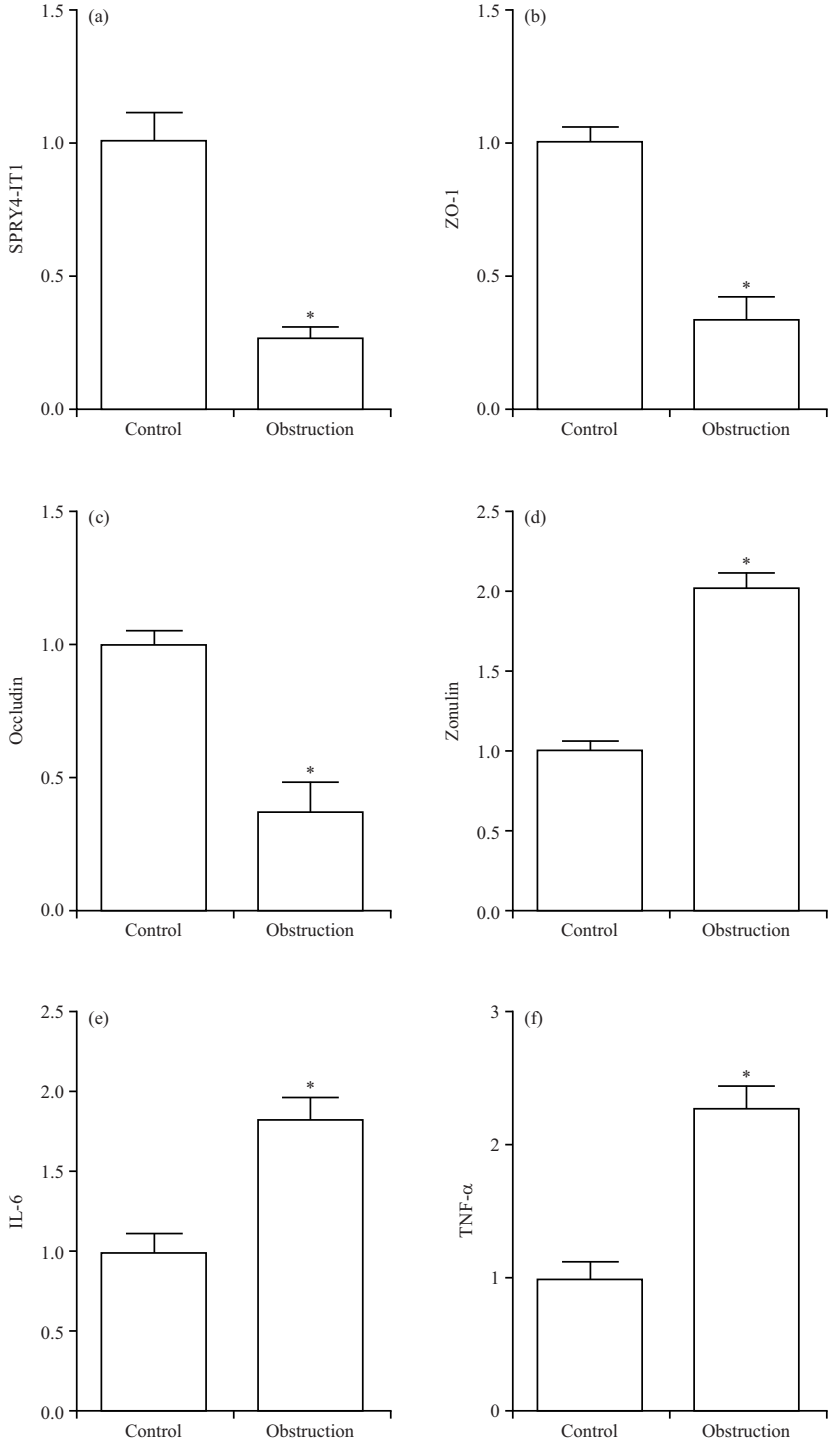

Fig. 5(a-f): Detection of the clinical colon and serum samples of acute stercoral obstruction patients for the detection of SPRY4-IT1, occluding and ZO-1 in colon tissues and zonulin, IL-6, TNF-α in serum samples, (a) Expression levels of SPRY4-IT1 decreased in the stercoral obstruction group, compared with paired obstruction-adjacent normal colon tissues, (b, c) Colonic occluding and ZO-1 expression levels decreased significantly in colonic obstruction patients, compared with that of paired obstruction-adjacent normal colon tissues and (d-f) Serum levels of zonulin, IL-6 and TNF-α increased in the acute stercoral obstruction group, compared with volunteer

*p<0.05 and Ten independent experiments were performed for each test

DISCUSSION

In this study, the therapeutic effects of matrine on intestinal barrier dysfunction via SPRY4-IT1 was investigated. Results indicated that matrine could activate SPRY4-IT1 pathway in Caco-2 cells, so as to protect intestinal epithelia barrier and higher expression of tight junction. Furthermore, mice model and clinical samples also indicated the protective effects of matrine on intestinal barrier and the key molecular of SPRY4-IT1.

Expression level of occluding in Caco-2 cells declined after the LPS stimulation, while matrine could inhibit the effects of LPS and higher the occluding expression. However, transfection of the SPRY4-IT1 inhibitor into Caco-2 cell line could significantly lower the occluding expression. According to the results, we hypothesized that matrine could protect intestinal barrier from dysfunction through SPRY4-IT1. It is reported that lncRNA SPRY4-IT1 protected intestinal epithelial barrier function by modulating the expression levels of tight junction proteins. However, SPRY4-IT1 has not been promoted as an important molecular mechanism during the protective process of intestinal barrier by matrine. In this report, transfection of SPRY4-IT1 inhibitor into Caco-2 cells could higher P38MAPK expression, compared with the single matrine group. In contrast, the expression level of P38 MAPK declined in Caco-2 cells after SPRY4-IT1 precursor transfection. In addition, we also verified the protective role of matrine and SPRY4-IT1 in the DSS induced mice colitis model and clinical samples of stercoral obstruction patients. Results indicated that SPRY4-IT1 might be one of the signaling pathways during the protective process of intestinal barrier function, by lowering the intestinal permeability *in vitro* and *in vivo*. Furthermore, matrine could also inhibit the inflammatory related factors and maintain the tight junction-associated proteins, such as occluding and ZO-1. Furthermore, clinical samples of stercoral obstruction patients indicated that IL-6 and TNF-α were both increased significantly, while SPRY4-IT1 and intestinal barrier related proteins, including and ZO-1, were decreased, compared with control.

One study about SPRY4-IT1 showed that it was activated by NF-kappaB/p65 to inhibit TCEB1 expression and subsequently upregulated HIF-1alpha, via STAU1-mediated degradation during cancer metastasis[23]. Most of the research focused on the mechanism of different carcinoma[24-26]. Only one study about SPRY4-IT1 found its protecting effects on the intestinal barrier and TJ[8], indicating that patients with increased gut permeability showed a decreased SPRY4-IT1 expression level[8]. In this study, further investigated was done on the key role of SPRY4-IT1 in the protective effects of matrine on intestinal barrier function and found that matrine could protect intestinal barrier from dysfunction via SPRY4-IT1 signaling. SPRY4-IT1 could be a potential target to treat intestinal barrier dysfunction.

One limitation of this study may be that we still could not launch a clinical trail to further guarantee the therapeutic effects of matrine on the intestinal barrier and its regulative effects on SPRY4-IT1. Whether there are some middle molecular mechanisms and pathways, or other key molecular should also be further studied as well. Further studies and high quality clinical trials would be needed to verify the related mechanism.

CONCLUSION

In conclusion, matrine could protect intestinal barrier from dysfunction via SPRY4-IT1 signaling. The molecular mechanism may be that matrine might inhibit SPRY4-IT1/P38MAPK pathway and higher the expression level of TJ proteins.

SIGNIFICANCE STATEMENT

Intestinal barrier dysfunction becomes more and more popular recent years and it is associated with many clinical diseases, such as inflammatory bowel disease, gut obstruction and so on. However, no efficient biomarkers and therapeutic drugs of intestinal barrier dysfunction have been found till now. In current study, a new molecular of SPRY4-IT1 for diagnosis of intestinal barrier dysfunction was promoted and also a new therapeutic drug of matrine for the treatment.

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