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Original Article

Berberine Enhances Intestinal Mucosal Barrier Function by Promoting Vitamin D Receptor Activity*

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ABSTRACT **Objective**: To evaluate if berberine can act on vitamin D receptors (VDR) and thereby regulate the expression of tight junction proteins (TJPs) in irritable bowel syndrome-diarrhea-predominant (IBS-D) rats. **Methods**: The newborn rats were induced into IBS-D rat model via neonatal maternal separation combined with acetic acid chemical stimulation. After modeling, the model was evaluated and rats were divided into the control group and berberine treatment groups (0.85, 1.7 and 3.4 mg/kg, once a day for 2 weeks). The distal colon was obtained and colonic epithelial cells (CECs) were isolated and cultured after IBS-D model evaluation. The vitamin D receptor response element (VDRE) reporter gene was determined in the CECs of IBS-D rats to analyze the effect of berberine on the VDRE promoter. VDR overexpression or silencing technology was used to analyze whether VDR plays a role in promoting intestinal barrier repair, and to determine which region of VDR plays a role in berberine-regulated intestinal TJPs. **Results**: The IBS-D rat model was successfully constructed and the symptoms were improved by berberine in a dose-dependent manner ($P<0.05$). The activity of VDRE promoter was also effectively promoted by berberine ($P<0.05$). Berberine increased the expression of TJPs in IBS-D CECs ($P<0.05$). VDR expression was significantly increased after transfection of different domains of VDR when compared to normal control and basic plasmid groups (all $P<0.05$). RT-qPCR and Western blot results showed that compared with the blank group, expressions of occludin and zonula occludens-1 were significantly higher in VDR containing groups (all $P<0.05$). Berberine plus pCMV-Myc-VDR-N group exerted the highest expression levels of occludin and zonula occludens-1 ($P<0.05$). **Conclusion**: Berberine enhances intestinal mucosal barrier function of IBS-D rats by promoting VDR activity, and the main site of action is the N-terminal region of VDR.

KEYWORDS berberine, Chinese medicine, irritable bowel syndrome-diarrhea-predominant, vitamin D receptors, intestinal mucosal barrier, tight junction proteins

Irritable bowel syndrome (IBS) is a globally recognized refractory functional bowel disease characterized by abdominal pain or abdominal discomfort accompanied by changes in bowel habits. Its main characteristics are recurring and prolonged and difficult to heal.[1] As the main sub-type of IBS, diarrhea-predominant IBS (IBS-D) accounts for 39.0%–61.9% of all IBS patients.[2] Evidence suggests that the pathogenesis of IBS-D involves changes in intestinal mucosal permeability,[3] gastrointestinal motility,[4] neuroendocrine disorders,[5] visceral hypersensitivity reactions[6] and brain-gut axis abnormalities.[7] Our previous study also confirmed that the pathogenesis of IBS-D is also closely related to the increase in intestinal permeability.[8]

Berberine is a yellow alkaloid in numerous plants, especially *Coptidis Rhizoma*, a traditional Chinese medicine.[9] It is widely used as an antimicrobial in the treatment of dysentery and diarrhea.[10,11] Our previous study has also confirmed that berberine has a function of protecting intestinal epithelial tight junction proteins

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(TJPs) and repairing damaged intestinal epithelial barrier in IBS-D rats.[12]

Vitamin D (Vit D) is a sterol derivative and fat-soluble vitamin that is closely related to body's calcium and phosphorus metabolism and bone calcification.[13] Recent studies have found that Vit D also appears to exert anti-inflammatory and immunomodulatory effects.[7,14,15] About 30% to 50% of the world's population has Vit D deficiency, and studies have shown that IBS patients suffering from osteoporosis are also significantly higher than the general population, and the incidence is similar to the incidence of gastrointestinal diseases.[7,16] The homeostasis of intestinal mucosal barrier is affected by Vit D, and mucosal damage usually appeared in patients with Vit D deficiency.[7,17,18] Studies have shown that Vit D supplementation maybe a safe option to improve the quality of life and symptoms of IBS-D patients.[7,19,20] However, the potential relationship between Vit D deficiency and IBS-D remains to be determined.

Vit D receptor (VDR) exists in the epithelial cells of gastrointestinal tract, which plays an important role in maintaining the integrity of intestinal mucosal barrier[18,21] and reducing mucosal inflammation.[22] Our previous study has shown that berberine exerts therapeutic effects by repairing damage to the structural integrity of colonic epithelium and increases occludin, claudin-1, zonula occludens (ZO)-1 and F-actin to improve epithelial tight junctions.[12] However, whether berberine can act on VDR and thereby regulate the expression of TJPs is not yet clear. Therefore, we used VDR response element (VDRE) reporter gene model *in vitro* in IBS-D rat colonic epithelial cells (CECs) and investigate the effect of berberine on colonic epithelial permeability through VDRE promoter by siRNA cell transfection and luciferin activity measurement. We then transfected different truncated VDR into CECs. Transepithelial resistance (TER) and paracellular flux measurements were used to detect the interaction between berberine and different VDR truncates on intestinal epithelial permeability. It would further determine which domain of VDR may be mainly responsible for the effect of berberine on IBS-D.

METHODS

Establishing IBS-D Rat Model

Totally 40 specific pathogen-free Sprague-Dawley pregnant rats, weighing 300±10 g, 10-week-old, were purchased from the Experimental Animal Center of Guangzhou University of Chinese Medicine (SCXK2019-0085, Guangzhou, China). They were adaptively fed with free eating and drinking. When the pregnant rats gave birth, the born rats (male or female) were randomly divided into the control and model groups. Neonatal maternal separation combined with acetic acid chemical stimulation was used to establish a classic IBS-D rat model.[23] From the 2nd to 14th day after the pups were born, the pups were separated from the lactating mother from 9:00 a.m to 12:00 a.m every day, and the mother rats and their pups were placed in the same cage during the rest of the time. From the 15th to the 30th day, the pups were treated with enema after being lightly anaesthetized with ketamine (75 mg/kg, lot No. 20203609, Zhejiang Jiuxu Pharmaceutical, Co., Ltd., China) and diazepam (5 mg/kg, lot No. 171225-200302, Shanghai Longlei Biotchnology Co., Ltd, China), and 0.5 mL of 0.5% acetic acid aqueous solution was applied to the pups' colon (approximately 2 cm from the rat anus) through a 1 mm epidural catheter. And 30 s after the enema, the colon was washed with an equal volume of phosphate buffered saline. The mother rats and their pups in the control group were kept in the same cage, and the same volume of normal saline was used for enema every day for the same period (15th to 30th day). After 14 days of recovery, 20 rats in each group were randomly selected for model evaluation on day 44 and sacrificed by cervical dislocation on the 45th day. The schematic diagram of IBS-D modeling is shown in Figure 1A.

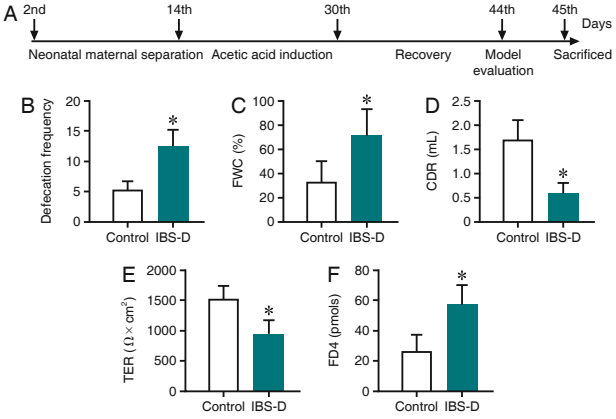

Figure 1. Successful Establishment of IBS-D Rat Model (*n*=20)

Notes: FWC: fecal water content; CDR: colorectal dilation response; TER: transepithelial resistance; *P<0.05 *vs*. control group

All animal studies were conducted in Laboratory Animal Center of Guangzhou University of Chinese

Medicine. And all animals received humane care in accordance with the guidelines set by the Care of Experimental Animals Committee of Guangzhou University of Chinese Medicine. Additionally, the present study was approved by the Ethics Committee of Guangzhou University of Chinese Medicine.

Treatment of Berberine

After confirmation of IBS-D model establishment, rats were treated with berberine Powder (B612348, 20 mg, Shanghai Boer Chemical Reagent Co., Ltd., China) for 2 weeks. Berberine was dissolved in saline solution before injection and the volumes of injections were adjusted to 0.85, 1.7, and 3.4 mg/kg, which was administered once daily for 2 weeks consecutively. The rats in the control group did not receive berberine treatment. The efficacy was evaluated after 2 weeks of treatment. After efficacy evaluation, the distal colon was obtained and CECs were cultivated.

Food Intake

After 2 weeks of treatment, the IBS-D rats were allowed to eat and drink freely. The rats were fed at 8:00 am and recorded the food weight as m_0. At 8:00 am of the next day, the leftover food was weighed and recorded as m_1. Food intake of each group in 24 h was calculated (m_0–m_1).

Fecal Water Content and Defecation Frequency

Based on our previous research report,[24] fecal water content (FWC) and defecation frequency detection were used to assess the permeability of the intestinal mucosa and the dynamic function of colon. In brief, the rats were placed in a metabolic cage for 24 h without deprivation of food and water. Stools were collected within 24 h, and fecal particles are counted and weighed (m_0). The fecal pellets were dried in an oven and weighed again (m_1). The value of FWC equals to (m_0–m_1)/m_0. Defecation frequency was the average number of fecal particles per hour.

Colorectal Dilation Response

The rats were fasted for 12 h without deprivation of drinking water, and then were lightly anaesthetized with ketamine (75 mg/kg) and diazepam (5 mg/kg), a 24-gauge catheter connected to a sphygmomanometer was inserted into the rat's colon to a depth of 8 cm from the anus. After the rats were awoken from anesthesia and adapted for 30 min, water was injected into the catheter, leading to colorectal dilation response (CDR). When the abdominal withdrawal reflex (AWR) score was 3, the amount of injected water was calculated as the degree of CDR. The AWR score was measured based on the criteria according to the previous study,[24] by 2 independent observers using a double-blind method. To obtain accurate measurement results, the CDR experiment was repeated 3 times at 2-min intervals.

Stool Consistency Score

Stool consistency score was performed one day after treatment, and the evaluation was mainly based on the Bristol Stool Scale.[25] Type 1: separate hard lumps, like nuts (hard to pass); Type 2: sausage-shaped, but lumpy; Type 3: sausage-shaped, but with cracks on the surface; Type 4: sausage- or snake-like, smooth, and soft; Type 5: soft blobs with clear-cut edges (easy to pass); Type 6: fluffy pieces with ragged edges, mushy; Type 7: watery, no solid pieces (entirely liquid). Type 1 stool was counted as 1 point, type 2 stool was counted as 2 points, and so on. The average value was taken as the stool consistency score of each group.

Isolation and Culture of CECs and TER Measurement

The culture process of CECs has been described in our previous research.[26] Simply, the distal colons were cut into small pieces, washed, and digested with 0.1% collagenase Ⅰ and hyaluronidase to separate the CEC clusters. The cells were collected, washed, counted, and cultured at 1×10^6 cm^2 on 150 mm cell culture dish (Costar, Corning, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) nutrient (Life Technologies, Shanghai, China) supplemented with 10% FBS (Gibco, USA), 1% penicillin/streptomycin (Life Technologies) and 16 mmol/L Hepes (Life Technologies) in a 37 ℃, 5% CO_2 incubator. When CECs adhered to the dishes, they were collected and seeded on 0.4-μm, 0.33-cm^2 polyester Transwell inserts (Costar, Corning, USA) at a density of 10^5 cells per Transwell. Mediums were refreshed every other day. Once CECs grew to complete confluence, epithelial integrity was evaluated by using TER measurements with an EVOM/Endohm (WPI, Sarasota, USA). The experiment was repeated 3 times to obtain the average value.

Paracellular Flux Measurements

Intestinal epithelial mucosal permeability

was used as a surrogate marker for the integrity of the mucosal barrier. In this study, fluorescein isothiocyanate-dextran 4 kDa (FD4; Sigma-Aldrich, USA) was used for measurement. The CECs were treated with or without berberine (Shanghai Shifeng Biotechnology Ltd., China) for 24 h, then FD4 (2 mg/mL) was added to the culture medium, and the intensity of fluorescein isothiocyanate in the basolateral fluid was measured with a fluorescence reader (FLUOstar Omega; BMG Labtech, Ortenberg, Germany). FD4 concentration was calculated and is expressed in picomoles (pmols).

Plasmids, siRNA Cell Transfection and Assay for Luciferase Activity

The VDRE promoter Luc vector and pGL3-Basic vector were constructed as previously described.[27] The plasmids of VDR with different functional domains (pCMV-Myc-Basic, pCMV-Myc-VDR, pCMV-Myc-VDR-N, pCMV-Myc-VDR-C) were kindly provided by Dr. HUANG Bing-ren (National Laboratory of Medical Molecular Biology, Chinese Academy of Medical Sciences & Peking Union Medical College, China). The RNA oligoribonucleotides (si-OCLN, si-ZO-1, VDR cDNA, and VDR siRNA) were synthesized from the Genepharma Corporation (China) as previous described.[8,27] VDR siRNAs were transfected into cells using Lipofectamine 2000 (11668030, Invitrogen, USA), according to the manufacturer's protocol.

The effects of berberine on VDRE promoter activity were studied by Dual Luciferase Assay. Vitamin D3 (VD3, VIT-013N, 100 mg, AccuStandard, Beijing Bailingwei Technology Co., Ltd., China) and different concentrations (1, 3, 30, 100 μg/mL) of berberine were dissolved in DMSO, respectively. The VDRE promoter reporter was transfected to the CECs of IBS-D rats and they then received different concentrations of berberine, respectively. The blank control group did not adopt any intervention measures, while VD3 was used as the positive control, due to its effectiveness in promoting the activity of VDRE promoter. Twenty-four hours later, a total of 10 μL of cell lysate was added to the cells, and the luciferase activity of each group was measured using a dual luciferase assay kit (E1910, Promega, Madison, USA). Renilla luciferase activity was standardized to detect Renilla luciferase activity of CECs.

After the optimal concentration of berberine was determined, we transfected CECs with VDRE promoter reporter and received either or not 30 μg/mL of berberine for 6, 12, 18, and 24 h again to determine the optimal time point. IBS-D rat CECs were transfected with si-OCLN, si-ZO1, and si-VDR and treated with 30 μg/mL of berberine for 18 h to evaluate the effective of permeability in CECs.

Real-Time Quantitative PCR

Real-time quantitative PCR (RT-qPCR) detection was performed based on the standard procedure we previously reported.[8] In brief, the concentration of the sample was adjusted to 50 ng/μL, and the PrimeScript RT Master Mix (RR036Q, TaKaRa, USA) was used to reverse transcribe the extracted RNA into complementary DNA (cDNA). RT-qPCR detection was performed on the ABI Prism 7500 PCR system (7500 Fast, Applied Biosystems, USA) using Platinum SYBR Green RT-qPCR SuperMix-UDG kit (C11744-500, Life Technology, USA). GAPDH was used as an internal reference. The primers were designed as follows: OCLN: 5'-AAGACGATGAGGTGCAGAAG-3' (forward) and 5'-GTGAAGAGAGCCTGACCAAA-3' (reverse); ZO-1: 5'-GGAGAGGTGTTTCGTGTTGT-3' (forward) and 5'-ACTGCTCAGCCCTGTTCTTA-3' (reverse); GAPDH: 5'-AGGTCGGTGTGAACGGATTTG-3' (forward) and 5'-GGGGTCGTTGATGGCAACA-3' (reverse). The mRNA expressions of OCLN and ZO-1 was calculated by the $2^{-\triangle\triangle Ct}$ method.

Western Blot Analysis

Western blot detection follows the standardized procedure we previously reported.[8] Primary antibodies include: OCLN (sc-133256, Santa Cruz), ZO-1 (sc-33725, Santa Cruz) and GAPDH (sc-47724, Santa Cruz). The blot was analyzed by ImageJ (v1.8.0; National Institutes of Health).

Statistical Analysis

SPSS 23.0 was used for statistical analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and one-way ANOVA analysis and Tukey's multiple comparison test were used to analyze differences among groups. The threshold of statistical significance was expressed as $P<0.05$.

RESULTS

Successful Establishment of IBS-D Rat Model

Compared with the control group, the defecation frequency and FWC values of the rats in the IBS-D

model group increased significantly (both $P<0.05$), while the CDR in the IBS-D group was remarkably decreased ($P<0.05$, Figures 1B–D). In addition, the TER in the IBS-D group was remarkably decreased, while the FD4 value was significantly up-regulated when compared with the control group ($P<0.05$; Figures 1E and F). The above data suggested that the IBS-D rat model was constructed successfully.

Berberine Improved Indisposed Symptoms in IBS-D Rats

After treating with 0.85, 1.7 and 3.4 mg/kg berberine, IBS-D rats ingested more food than the control group ($P<0.05$, Figure 2A). The defecation frequency and stool consistency score decreased significantly in the 0.85, 1.7, 3.4 mg/kg berberine groups than the control group ($P<0.05$, Figures 2B and 2C). Similarly, the FWC values decreased significantly in berberine groups (0.85, 1.7, 3.4 mg/kg) compared with the control group ($P<0.05$, Figure 2D).

Berberine Increased Activity of VDRE Promoter and Improved Permeability of CECs

The optimal VDRE promoter activity concentration was 30 μg/mL, which was significantly higher than the other groups ($P<0.05$, Figure 3A). And the optimal time point of VDRE promoter activity of the CECs after berberine treatment was 18 h ($P<0.05$, Figure 3B). Hence, 30 μg/mL berberine treatment for 18 h was used for subsequent experiments.

The TER in the IBS-D group was remarkably increased ($P<0.05$), while the FD4 value was significantly downregulated when compared to the control group ($P<0.05$). However, there were no significant differences among si-OCLN, siZO-1, si-VDR groups, and the control group ($P>0.05$, Figures 3C and 3D).

Berberine Promoted Expression of TJPs on CECs and Required VDR

TER measurement revealed significantly higher TER in CECs treated with berberine (30 μg/mL) compared with CECs without berberine treatment ($P<0.05$, Figure 4A). Besides, RT-qPCR revealed that the mRNA expressions of OCLN and ZO-1 were significantly increased compared with CECs that were not treated with berberine ($P<0.05$, Figure 4B).

The Western blot results showed significantly increased expressions of OCLN and ZO-1 in the group with combination of VDR cDNA and berberine ($P<0.05$, Figure 4C). On the other hand, VDR siRNA markedly inhibited the expressions of OCLN and ZO-1 in CECs ($P<0.05$, Figure 4D). The results of RT-qPCR showed similar trends to Western blot results ($P<0.05$, Figures 4E and 4F).

N-terminal Region of VDR Is Responsible for Protective Action of Berberine in Intestinal Mucosal Barrier of IBS-D Rats

The functional regions of VDR fusion proteins were shown in Figure 5A. The VDR expression was significantly increased after transfection of different domains of VDR when compared to normal control and basic plasmid groups (all $P<0.05$, Figure 5B).

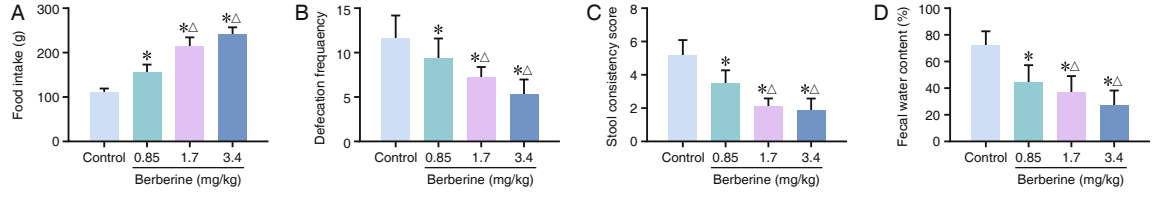

Figure 2. Berberine Improved the Symptoms of IBS-D Rats ($\bar{x} \pm s$, n=20)

Notes: *$P<0.05$ *vs*. control group; △$P<0.05$ *vs*. berberine group (0.85 mg/kg)

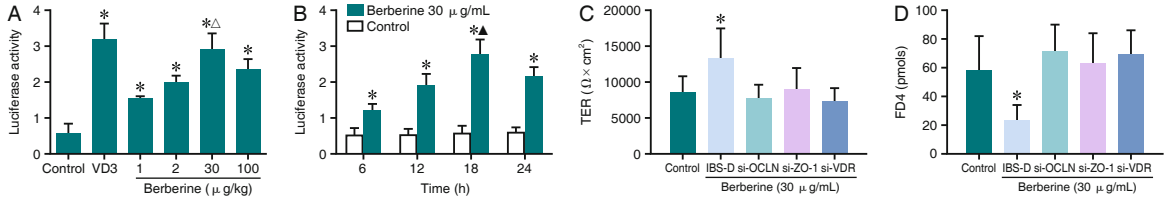

Figure 3. Berberine Increased VDRE Promoter Activity and Improved Permeability of CECs ($\bar{x} \pm s$, n=20)

Notes: *$P<0.05$ *vs*. control group; △$P<0.05$, 30 μg/mL berberine *vs*. the other dose berberine groups; ▲$P<0.05$, 18 h *vs*. other time points. VDRE: vitamin D reaptor response; CEs: colonic epithelial cells

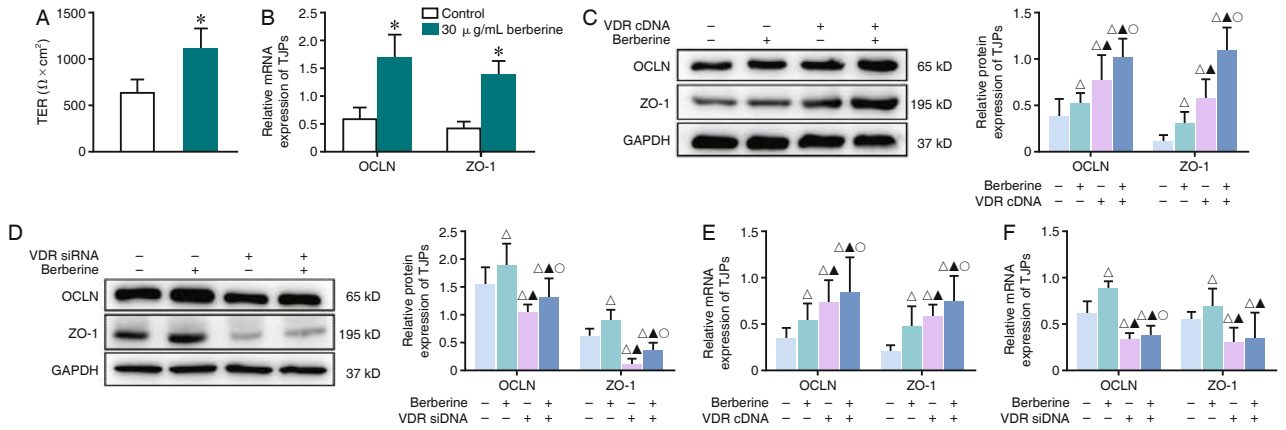

Figure 4. Berberine Promoted the Expression of TJPs on CECs and Required VDR

Notes: TER: transepithelial resistance; TJRs: tight junction proteins. *P<0.05 *vs*. 0 μg/mL berberine group; △P<0.05 *vs*. the group transfected with nothing and did not receive berberine treatment; ▲P<0.05 *vs*. the group transfected with nothing but received berberine treatment; ○P<0.05 *vs*. the group transfected with VDR cDNA/siRNA but did not receive berberine treatment

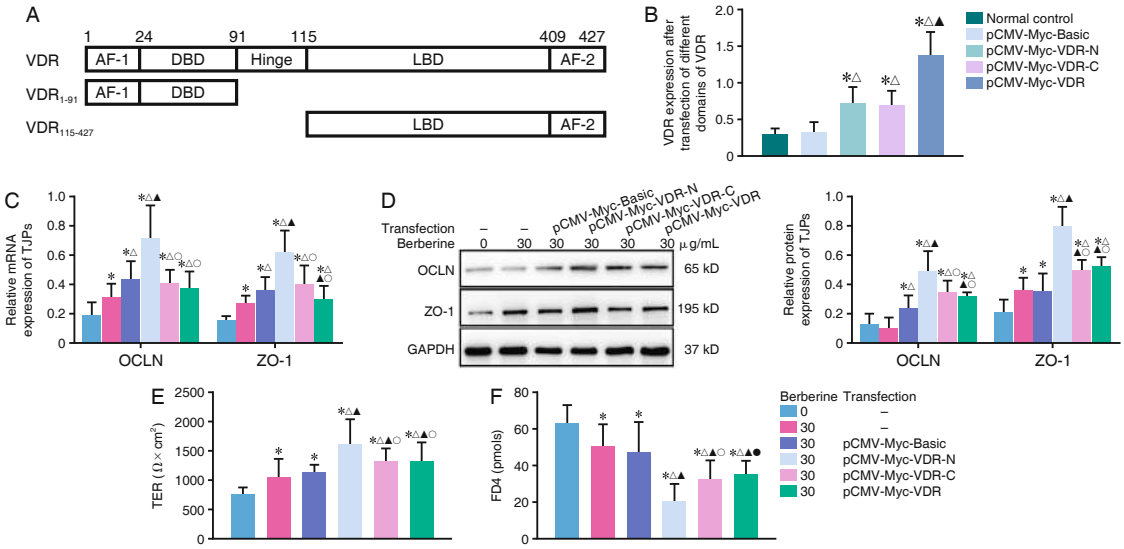

Figure 5. Berberine Protects Intestinal Mucosal Barrier Function through N-terminal Region of VDR

Notes: A: Functional regions of VDR fusion proteins. B: *P<0.05 *vs*. normal control group; △P<0.05 *vs*. pCMV-Myc-Basic group; ▲P<0.05 *vs*. pCMV-Myc-VDR-C and pCMV-Myc-VDR-N groups. C–F: *P<0.05 *vs*. blank control group; △P<0.05 *vs*. berberine group; ▲P<0.05 *vs*. pCMV-Myc-Basic+berberine group; ○P<0.05 *vs*. pCMV-Myc-VDR-N+berberine group; ●P<0.05 *vs*. pCMV-Myc-VDR-C+berberine group

RT-qPCR showed that compared with the blank group, the expressions of OCLN and ZO-1 were significantly higher in VDR containing group (all P<0.05); of which berberine + pCMV-Myc-VDR-N group exerted the highest expression levels of OCLN and ZO-1 (P<0.05, Figure 5C). The Western blot results were consistent with RT-qPCR results (P<0.05, Figure 5D). Besides, the TER was remarkably increased while FD4 was significantly decreased in berberine + pCMV-Myc-VDR-N group compared with berberine + pCMV-Myc-VDR group (all P<0.05, Figures 5E and 5F).

DISCUSSION

Our previous research has confirmed that IBS-D is related to increased permeability.[8] The permeability of intestinal epithelial cells is closely related to tight junctions. Tight junctions are highly polarized gates which are located at the most apical on the lateral membrane.[28] The claudins and OCLN comprise two of the integral membrane components of tight junction, where they interact with proteins of the ZO family.[29] This interaction in turn links the tight junction to the actin cytoskeleton, which controls the flux of fluids, proteins, and even ions across sheets of endothelial or epithelial cells.[28] These barriers function in a range of tissues, including the vasculature of the central nervous system, kidney, and gut epithelium.[28] It can effectively improve the barrier function of the intestinal mucosa by inhibiting inflammation.[4] In a recent study, we found that berberine can protect the tight junctions

of the intestinal epithelium and repair the damage to the intestinal epithelial barrier caused by IBS-D.[12] Studies have shown that one of the new functions of VDR is to maintain the skin barrier function,[30] and VDR seems to be closely related to the expression level of TJPs between intestinal epithelial cells and the permeability of intestinal mucosa.[17,18] When the intestinal mucosal barrier is destroyed, increased intestinal permeability can cause a decrease in the expression of VDR.[17,18] Berberine has been confirmed by our study that it participates in the protective function of intestinal mucosal barrier,[12] hence we hypothesized that VDR may be one of the direct mediators of berberine.

A series of experiments were performed to test this hypothesis. Interestingly, we found that berberine can effectively promote the activity of VDRE promoter. Evidence of VDR knockdown in IBS-D CECs supports that berberine's role in the IBS-D intestinal mucosal barrier requires the participation of VDR. VDR is the nuclear hormone receptor of the vitamin D endocrine system, which is composed of a C-terminal ligand binding domain, an intermediate hinge region, and an N-terminal DNA binding domain.[26] Its main function is to maintain the dynamic balance of calcium and phosphate and ensure bone development.[31] Recent studies have reported an increased risk of osteoporosis and osteoporotic fractures in patients with IBS-D.[32] Vitamin D deficiency can be seen in a variety of gastrointestinal diseases.[33] Research has also confirmed that low Vit D level is common in IBS-D patients.[34] Khalighi, et al[19] have strongly supported that VDR plays a direct role in promoting the therapeutic effect of IBS-D. $VDR^{-/-}$ mice can lead to abnormal Paneth cells and reduced autophagy, which may alter the clearance of bacterial infections and alter mucosal defenses.[18] Study also found that the absence of VDR can cause enteral malnutrition in mice, which is an important cause of various intestinal diseases.[18] Therefore, there is promising strategy that targets the VDR to regulate intestinal health. In addition, we conducted research through a series of VDR deletion mutants and found that the N-terminal region of VDR is responsible for the role of berberine, which binds to lipophilic ligands to regulate the intestinal mucosal barrier and maintain intestinal health.

The results we obtained are also promising in many aspects of clinical application. First of all, the use of berberine, an active ingredient of CM that is widely used clinically in IBS-D, can reduce intestinal mucosal permeability and effectively protect mucosal barrier function.[35] Secondly, the importance of VDR in the protective mechanism of IBS-D intestinal mucosa was verified at the cellular level, and it plays a key role in regulating intestinal mucosal tight junction structure, intestinal environment homeostasis, and defense against invading pathogens.[36,37] Furthermore, this study explored how berberine regulates VDR to affect intestinal mucosal barrier function from a pharmacological point of view. The results of this study provide an important basis for CM targeted therapy. It was found that the active ingredients of CM had therapeutic effect through biological information transmission.[38] Similarly, we found that the protective effect of berberine on intestinal mucosal epithelial cells was determined by VDR in this study. The results found the specific regions where VDR plays a corresponding role, providing a new idea for the repair and treatment of intestinal mucosal barrier function of IBS-D, and laying a foundation for the development of gene target research that can improve the therapeutic effect of IBS-D.

However, the possible mechanisms by which berberine binds to the N-terminal region of VDR and how it affects the specific activity of CECs still need to be further investigated. Due to the low absorption rate of berberine in the intestine, the relevant mechanism may be related to the interaction of intestinal flora. A study on high-fat diet fed obese mice showed that astragalus polysaccharide combined with berberine can regulate intestinal tract microbiota, increasing *Bacteroides* abundance to treat high-fat diet-induced obesity in mice.[39] Berberine may also affect metabolites by regulating intestinal flora, and alleviate symptoms in type 2 diabetic rats.[40] This study may provide us an important clue for further exploration. The targeted therapeutic effect of IBS-D is likely to be based on the interaction between the metabolites produced by intestinal flora and the N-terminal region of VDR, thereby improving the intestinal epithelial cell barrier function. Hence, the strategy that increasing the absorption of Vit D, resulting in increased expression of TJPs, will finally repair the intestinal mucosal barrier function. However, the absorption and uptake of berberine by intestinal epithelial cells and how VDR increases the expression of TJPs need to be further explored.

In conclusion, we report that berberine treatment can activate VDR activity and reduce intestinal mucosal permeability. The N-terminal region of VDR is the main regulatory region of berberine in regulating the intestinal mucosal barrier of IBS-D. Understanding how berberine enhances VDR signal transduction and improves the intestinal mucosal barrier function will enable berberine to be used effectively. This study may also provide a new idea for IBS-D CM targeted therapy.

Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

The authors are grateful to Dr. HUANG Bing-ren who provided help with plasmids used in this study. Huang YQ and Hou QK conceived and designed the project and prepared the manuscript. Liu JL, Chen GX, Shen DT and Zhu W performed the experiments. Chen XL, Liu FB and Hou QK analyzed the results. All authors read and approved the manuscript for publication.

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