Int J Clin Exp Pathol 2019;12(7):2786-2792
www.ijcep.com /ISSN:1936-2625/IJCEP0096028

Original Article

Characteristics of intestinal microflora involved insacral nerve stimulation affecting visceral hypersensitivity

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Received April 25, 2019; Accepted May 24, 2019; Epub July 1, 2019; Published July 15, 2019

Abstract: Objective: This study was to investigate the effect of intestinal flora-intestinal-brain on the visceral sensitivity of normal rat and visceral hypersensitivity model rats, and to explore the effect of intestinal flora-intestinal-brain axis on visceral hypersensitivity in rats. Methods: Sixty SD male newborn rats were randomly divided into 4 groups according to the random number table: group A, group B, group C, and group D, with 15 rats in each group. 15 sterile SD newborn rats, numbered E group. Group A: normal model group; group B, group C, group D, group E, all used acetogen enema sensitization method to establish a visceral hypersensitivity model. Group C was given the vancomycin antibiotic before the model group and in group D sacral nerve stimulation (SNS) was given after modeling. At the end of the treatment period, the visceral sensitivity, intestinal flora expression, expression of NGF, TrKA, NF-κB, TRPV1, pTRPV1, IL-1β, IL-10, IL-22, TNF-α, 5-HT, and γ-GABA were measured in each group. Results: (1) The VMR values of the sterile rat model group were significantly different from those of the model group and the SNS stimulation model group (P<0.01). The VMR values of the antibiotic model group were statistically significant compared with the SNS stimulation model group (P<0.01). (2) There was no bacterial growth in the sterile rat model group. The expression levels of the four bacterial groups were significantly different between the antibiotic model group and the SNS stimulation model group (P<0.01). (3) The expression of NGF and TrKA in the SNS stimulation model was higher than that in the antibiotic model group (P<0.05). The expression of NF-κB and pTRPV1 was lower than that in the model group (P<0.05). The NGF, TrKA, NF-κB, and pTRPV1 were hardly expressed, which was significantly lower than the other groups (P<0.05). (4) There was no significant difference in the content of each index between the normal model group and the antibiotic model group (P > 0.05), IL-10 and 5-HT levels in the normal model group, the sterile rat model group, and the antibiotic model. There was no significant difference between the group and the sterile rat model group (P > 0.05). The difference was statistically significant (P<0.01). Conclusion: The neurotransmitter produced by the intestinal flora can bind to the receptor TrkA and the translocation channel TRPV1 of intestinal tissue and CNS tissue, causing intestinal sensitivity changes.

Keywords: Intestinal microflora, SNS, visceral hypersensitivity

Introduction

Irritable bowel syndrome (IBS) is the most common digestive tract disease. The prevalence of IBS in the general population is 3-22% [1-3], which seriously affects the quality of life of patients and consumes a lot of medical resources. The mechanism of IBS is still unclear. Visceral hypersensitivity is considered to be one of the main causes of IBS. Visceral hypersensitivity is closely related to neuroplasticity in pain pathways of central, peripheral, and enteric nervous system (ENS) [4]. At present, the treatment of IBS is mainly to improve the symptoms. The curative effect is not currently satisfactory and the symptoms often recur. Exploring the visceral hypersensitivity mechanism of IBS and looking for new treatment ideas are the research hotspots of scholars.

Sacral nerve stimulation (SNS) is a type of peripheral nerve regulation. It was initially used for the treatment of urinary incontinence and retention. In 1995, it was used by Matzel and others for minimally invasive treatment of fecal incontinence [5]. SNS is more and more widely used in the treatment of bladder dysfunction, fecal incontinence, and some intractable constipation because of its minimally invasive, safe, effective, and economical characteristics [6-8]. However, the high sensitivity of SNS to IBS or viscera has rarely been reported. Fassov J performed SNS on 21 DIARRHEA-TYPE IBS patients and found that some patients' symp-

toms and quality of life improved after treatment [9]. Langlois L reported that anorectal dilatation (acute visceral hypersensitivity model) and SNS on normal SD rats could improve their visceral hypersensitivity induced by anorectal dilatation [10, 11].

The intestine and brain have biphasic regulation [12]. Neural network system regulating intestinal function includes two parts: internal nervous system and external nervous system, which coordinate with each other and regulate intestinal function together [11]. In recent years, more and more studies have begun to pay attention to the effects of intestinal microorganisms on the brain. It is believed that there is a close information exchange among intestinal microorganisms, intestinal microorganisms, and the brain. Therefore, the microorganism-intestine-brain axis is proposed [11, 13]. The vagus nerve is the main nerve anatomical basis of this axis, the nerve with the longest journey, and the widest distribution in the brain nerve. The efferent fiber terminals of the vagus nerve mainly form synaptic connections with the postganglionic neurons in the intestinal myenteric plexus, which can transmit a variety of intestinal information to the brain [14]. The central nervous system controls the intestinal tract through the autonomic nervous system (including the vagus nerve) and thus changes local intestinal environments, such as intestinal microbial composition [15]. It is believed that the microorganism-gut-brain axis can interact through the immune pathway, such as intestinal microorganism, influencing the level of anti-inflammatory/pro-inflammatory factors in circulation through immune response. This, in turn, affects the function of central nervous system [16, 17]. The imbalance of intestinal microorganism and the excessive growth of intestinal bacteria will activate innate immunity. This adaptive immune response may also be caused by the cross immunization of bacterial protein and human protein. Activation of the immune response will increase intestinal epithelial permeability, leading to local and central nervous system inflammatory response [18, 19]. In addition, the neuroendocrine mechanism plays an important role in the interaction of the microorganism-intestine-brain axis. Intestinal bacteria can synthesize various neurotransmitters and neuromodulators, such as 5-HT, γ-GABA, dopamine, short-chain fatty acids, etc. These neurochemicals play a signal transduction role between bacterial cell membranes and have relative specificity [11, 20]. The disorder will lead to the obstruction of signal transmission, which will have an impact on the central nervous system. The microbial-intestinal-brain axis plays an important role in regulating intestinal motility, secretion, and visceral stimulation.

There are at least 40 genera of intestinal microflora in most people, including more than 400 strains, mainly specialized anaerobic bacteria. Different intestinal microflora maintains their respective proportions in the intestine, maintains intestinal function, and protects human health. In some cases (irregular life, intestinal infection, and overuse of antibiotics), the proportion of intestinal bacteria changes and the steady state is difficult to maintain, further developing into intestinal flora imbalance. Relevant studies have confirmed that intestinal flora imbalance plays an important role in the occurrence and development of IBS [21-23]. It is noteworthy that recent studies have shown that intestinal microorganisms also play an important regulatory role in central nervous system and brain function [24].

The visceral hypersensitivity animal model was established by acetic acid enema. The expression of intestinal flora including Bifidobacterium, Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus paracasei, NGF, TrKA, NF-κB, TRPV1, and pTRPV1 in rat dorsal root ganglion and colon tissues, and the expression of inflammatory factors IL-1β, TNF-α, IL-10, IL-22, and neurotransmitters 5-HT and γ-GABA were determined to explore the effect of intestinal flora-intestinal-brain axis on visceral sensitivity and its possible regulatory mechanism, so as to provide a new theoretical basis for the pathogenesis of IBS and provide new ideas for the treatment and drug development of IBS.

Materials and methods

Laboratory animals and groups

Sixty SD-grade male neonatal rats were fed at quiet, room temperature (22 + 2°C), constant humidity (40-50%), and 12/12 h circadian circulation. After one week of adaptive feeding, 60 healthy, clean SD rats were randomly divided into four groups: group A, group B, group C, and group D; with 15 rats in each group. At the same time, 15 sterile SD rats were purchased and fed under the same conditions, labeled group E.

Group A: Normal model group.

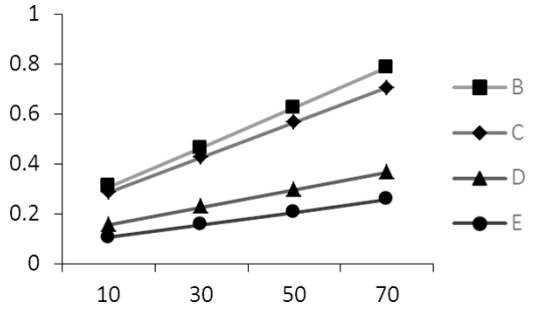

Figure 1. Trend diagram of visceral motor reflex values after rectal dilation in rats.

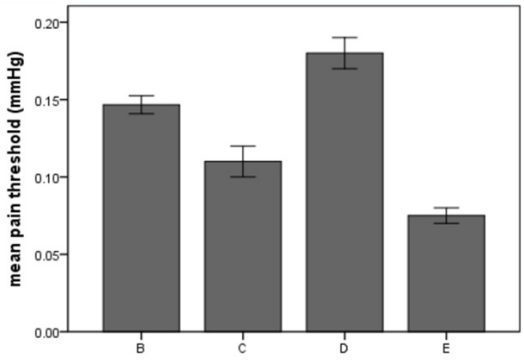

Figure 2. Mean pain threshold values of rats in each group.

Group B: The visceral hypersensitivity model was established by acetic acid enema sensitization. No treatment was given after the model was successfully established.

Group C: Vancomycin was given before the model was established and then the visceral hypersensitivity model was established by acetic acid enema sensitization.

Group D: Sacral nerve stimulation was given on the basis of the model group.

Group E: The visceral hypersensitivity model was established by acetic acid enema sensitization. No treatment was given after the model was successfully established.

Methods

At the age of 10 days, neonatal rats were fed with 0.5% acetic acid solution 0.2 mL through anus and 2 cm through intestinal perfusion; rats in group A were fed with 0.2 mL saline through anus and 2 cm through intestinal perfusion; rats in group C were given antibiotic vancomycin before modeling; rats in group D were given sacral nerve stimulation after successful modeling. Electrode implantation was performed at the age of 7 weeks in rats and the experiment began at the age of 8-12 weeks.

Referring to previous literatures, a method for detecting visceral motor response (VMR) was developed using colorectal dilatation (CDR). Under anesthesia (inhalation of 1.0-1.5% isoflurane), a self-made balloon was inserted into the rectum and descending colon through the anus of rats to 8 cm and the catheter was affixed to the tail of rats. Rats were placed in a restricted cage for 30 minutes. Colorectal dilatation (CDR) was performed by rapid filling of balloons under constant pressure monitored by a sphygmomanometer. The balloon was inflated at 10, 30, 50, and 70 mmHg for 20 seconds, and then relaxed for 2 minutes to draw the electromyogram (EMG) under different pressures. The VMR values of all rats were measured and calculated again after implantation of electrodes for 3 days and grouping treatment according to the above methods, which served as an index for evaluating visceral sensitivity.

DEGG-PCR was used to analyze the expression of intestinal flora in feces of rats in each group: Bifidobacterium, Lactobacillus rhamnosus, Lactobacillus acidophilus, and Lactobacillus paracasei; WB was used to analyze the expression of NGF, TrKA, NF-κB, TRPV1, and pTRPV1 in dorsal root ganglion and colon tissues; ELISA was used to analyze the contents of inflammatory factors IL-1β, IL-10, IL-22, TNF-α, and neurotransmitters 5-HT, γ-GABA in blood samples of mice.

Statistical methods

SPSS 18.0 statistical software was used to process and analyze the data and the measurement data was described by mean (+) standard deviation. Single factor analysis of variance was used for multi-group comparison and correlation analysis was used for correlation analysis. $P<0.05$ was statistically significant.

Results

Sensitivity of colon tissue in each group

As shown in **Figure 1**, VMR was used to assess the sensitivity of colon tissue in each group. Group E (sterile rat model group) had VMR values after 10, 30, 50, and 70 mmHg CDRs with group B (model group) and group D (SNS stimulation model). As shown in **Figure 2**, the differences were statistically significant ($P<0.01$);

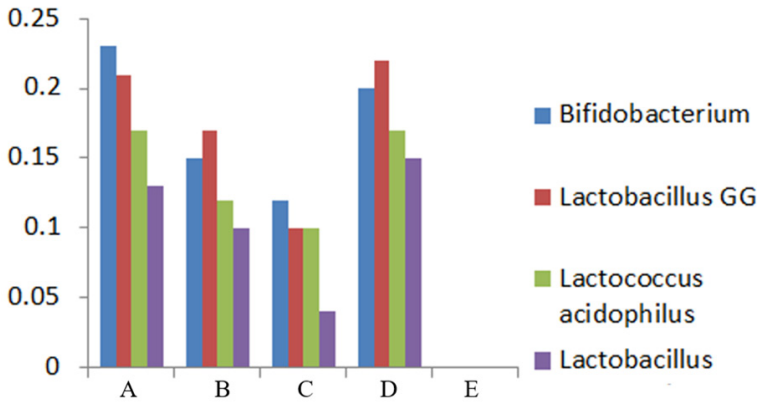

Figure 3. Expression of Bifidobacterium, Lactobacillus rhamnosus, Lactobacillus acidophilus, and Lactobacillus paracasei in feces of rats in each group.

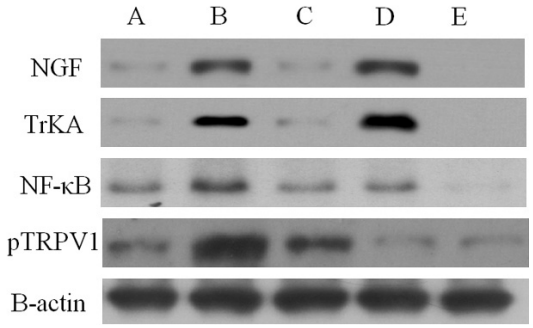

Figure 4. Expression of proteins in rats of each group.

the VSR values after the 10, 30, 50, and 70 mmHg CDRs in the C group (antibiotic model group) were significantly different from those in the D group (SNS stimulation model group) (P<0.01).

Expression of Bifidobacterium, Lactobacillus rhamnosus, Lactobacillus acidophilus, and Lactobacillus paracasei in feces of rats in each group

DEGG-PCR was used to analyze the expression of Bifidobacterium, Lactobacillus rhamnosus, Lactobacillus acidophilus, and Lactobacillus paracasei in the feces of rats in each group. As shown in **Figure 3**, there was no bacterial growth in group E (aseptic rat model group) and there was significant difference in the expression of four bacterial groups between group C (antibiotic model group) and group D (SNS stimulation model group) (P<0.01).

Expressions of NGF, TrKA, NF-kappa B, and pTRPV1 in rats of each group

As shown in **Figure 4**, the expression of NGF, TrKA, NF-kappa B, and pTRPV1 in group A (normal non-model group) was detected by western blotting. The expression of NGF, TrKA, NF-kappa B, and pTRPV1 in group B (model group) was high. The expression of NGF, TrKA, NF-kappa B, and pTRPV1 in group C (antibiotic model group) was low. The expression of NGF, TrKA, NF-kappa B, and pTRPV1 in group D (SNS stimulation model group) was high. The expression of NF-kappa B and pTRPV1 was higher than that in group C (P<0.05). The expression of NF-kappa B and pTRPV1 was lower than that in group B (P<0.05). The expression of NGF, TrKA, NF-kappa B and pTRPV1 in group E (aseptic rat model group) was almost not expressed, which was lower than that in other groups (P<0.05).

Contents of inflammatory factors IL-1β, IL-10, IL-22, TNF-α, and neurotransmitters 5-HT and γ-GABA in blood samples of rats in each group

The levels of inflammatory factors IL-1β, IL-10, IL-22, TNF-α, and neurotransmitters 5-HT, γ-GABA in blood samples of mice were analyzed by ELISA and the levels of inflammatory factors IL-1β, IL-10, IL-22, TNF-α, and neurotransmitters 5-HT and γ-GABA in blood samples of rats of each group were significantly different (P <0.01). As shown in **Figure 5**, the results showed that there was no significant difference in the contents of IL-10 and 5-HT between group A (normal model group) and group C (antibiotic model group) (P > 0.05). The contents of IL-10 and 5-HT in group A (normal model group) and group E (aseptic model group) and in group C (antibiotic model group) and group E (aseptic model group). There was no significant difference between the two groups (P > 0.05). There was significant difference between the other two groups (P<0.05).

Discussion

Irritable bowel syndrome (IBS) is a functional gastrointestinal disease characterized by repeated abdominal pain or abdominal discomfort accompanied by changes in defecation habits [25]. The incidence of IBS can reach 10%~20%, mainly in women, which can significantly reduce the quality of life of patients [26]. At present, the research on the pathogenesis of IBS mainly

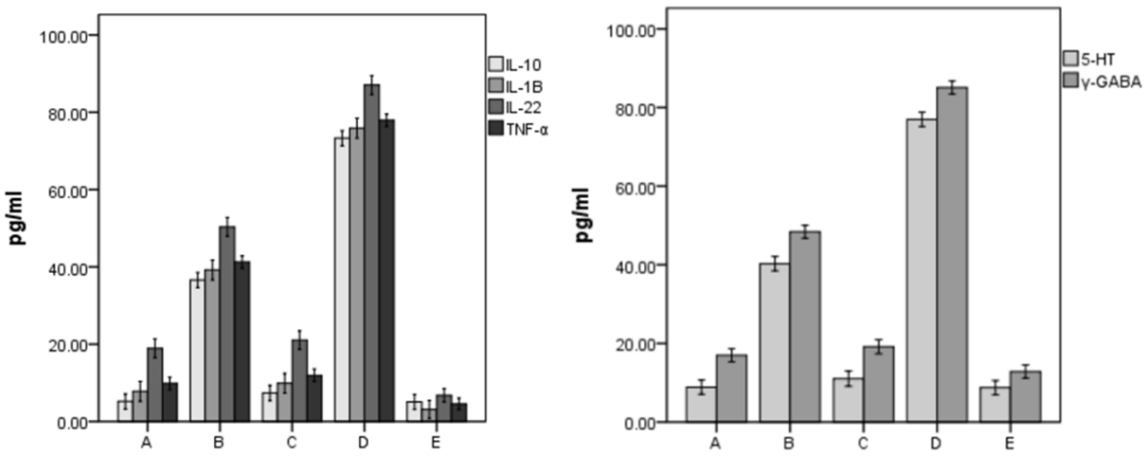

Figure 5. Contents of IL-1β, IL-10, IL-22, TNF-α, 5-HT and γ-GABA in rats of each group.

focuses on genetic susceptibility, psychosocial stress, visceral hypersensitivity, disorder of brain-gut axis regulation, intestinal flora imbalance, and so on. The structure, quantity, and distribution of intestinal flora in IBS patients were different from those in normal individuals. Codling found that the diversity of intestinal flora in IBS patients was lower than that in normal people [27]. The study also found that there was no significant difference in the composition of intestinal flora between IBS patients and intestinal mucosal flora, indicating that IBS had no effect on the composition of intestinal flora in different niches, but the composition of intestinal flora in IBS patients changed compared with normal people. Changes in the structure of intestinal flora, such as the decrease of symbiotic bacteria and the increase of pathogenic bacteria, can activate intestinal immune response. In addition, intestinal inflammation is associated with abnormal brain-gut axis regulation.

Intestinal glial cell proliferation and activation are the main sources of neurotrophic factors, such as nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), neurotrophic protein (NT-3) in inflammatory state, and play an important role in the formation of intestinal neuroplasticity. Mast cells release NGF, NT-3, and NT-4, which promote the proliferation of SP, CGRP, and TRPV1 positive nerve fibers. Studies [28-31] show that TRPV1, TRPA1, neurokinin receptor, protease-activated receptor, voltage-gated calcium channel, and sodium channel are related to visceral hypersensitivity. They participate in peripheral and central sensitization of IBS by increasing synaptic transmission efficiency and reducing visceral pain threshold. TRPV1 and TRPA1 cationic channels are highly expressed in ENS, DGR, and spinal dorsal horn, and their activities are enhanced. They mediate Na^+, Ca^{2+} currents and participate in the formation of afferent nerve excitability and visceral hypersensitivity at the peripheral and spinal levels [31].

This study found that the visceral sensitivity of the aseptic rat model group was the lowest ($P<0.01$), and the expression of bacteria in the model group was significantly different from that in the normal non-model group ($P<0.01$). The expression of histone NGF and TrKA in SNS stimulation model group was higher than that in the antibiotic model group ($P<0.05$). The expression of NF-kappa B and pTRPV1 was lower than that in the model group ($P<0.05$). The expression of histone NGF, TrKA, NF-kappa B, and pTRPV1 in aseptic rat model was almost not expressed, which was significantly lower than that in other groups ($P<0.05$). There was no significant difference in the contents of IL-1β, IL-10, IL-22, TNF-α, 5-HT, and γ-GABA between the normal model group and the antibiotic model group ($P > 0.05$). There was no significant difference in the contents of IL-10 and 5-HT between the normal model group and the aseptic rat model group, and between the antibiotic model group and the aseptic rat model group ($P > 0.05$). There was a significant difference between the other two groups ($P<0.05$).

In conclusion, we found that the neurotransmitters produced by intestinal flora can bind to the

receptor TrkA, which causes the opening of the transport channel TRPV1 of intestinal tract and CNS, and increases visceral sensitivity.

Acknowledgements

This study was approved by National Natural Science Foundation of China for Youth (81700470).

Disclosure of conflict of interest

None.

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