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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2016.06.003

Role of intestinal flora imbalance in pathogenesis of pouchitis

Xiao-Bo Feng[1,#], Jun Jiang[1,#], Min Li[1], Gang Wang[1], Jin-Wei You[1], Jian Zuo[2*]

[1]*Research Institute of General Surgery, Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu Province 210002, People's Republic of China*

[2]*State Key Laboratory of Infectious Disease Diagnosis and Treatment, First Affiliated Hospital of Medical School of Zhejiang University, Hangzhou, Zhejiang Province 310003, China*

ARTICLE INFO

Article history:
Received 15 May 2016
Received in revised form 16 Jun 2016
Accepted 21 Jun 2016
Available online 28 Jun 2016

Keywords:
Intestinal flora
Pouchitis
Bifidobacterium
Lactobacillus
Bacteroid

ABSTRACT

Objective: To discuss the role of intestinal flora imbalance in the pathogenesis of pouchitis.

Methods: The pouchitis rat model was established and the faeces sample and the mucous membrane sample were collected regularly, in which the bacterial nucleic acids were extracted for quantitative analysis of the intestinal flora in the samples through using the real-time quantitative PCR technique and high energy sequencing technology.

Results: The disorder phenomenon of the intestinal flora appeared at the 7th day of the experiment, and the pouchitis was presented at the 21st day of the experiment. At the 31st day of the experiment, compared to control group and non-pouchitis group, the quantity of *Bifidobacterium* and the *Lactobacillus* of the pouchitis model rats in the mucous membrane sample and the faeces sample were significantly decreased ($P < 0.05$), and the *Bacteroidetes*, *Faecalibacterium prausnitzii* and XIV *Clostridium leptum* subgroup in the mucous membrane of pouchitis were significantly decreased ($P < 0.05$). The IV *Clostridium coccoides* group was the main flora in the mucous membrane of pouchitis, the bacterial diversity of non-pouchitis group and control group was significantly higher than that of the pouchitis group ($P < 0.05$).

Conclusions: The intestinal flora imbalance is one of the factors that cause the incidence of the pouchitis; this study provides a clue of the pathogenesis and treatment direction of the intestinal inflammatory disease.

1. Introduction

The incidence rate of pouchitis of ulcerative colitis after the operation is 50%, and it will increase with the extension of time. There are 61% of the patients who are multiple attacked by this disease, and 5%–19% of the patients will develop into chronic pouchitis [1,2]. There are many similarities of the phenotype of the pouchitis, crohn disease and ulcerative colitis, which is that the individual predisposing gene is activated through the factors of infection and surroundings, and cause the abnormity of the tolerance of intestinal immune, hence leading to the continuous and sustained inflammatory reactions and damage of tissues [3–5].

Pouchitis is the intestinal inflammatory disease that can be expected, which is an uncommon model of intestinal inflammatory disease that can be follow-up researched from premorbid [6]. Therefore, pouchitis is seen as the research object to study and discuss the cause and effect relationships between the changes of the intestinal flora and the incidence of the inflammation, so as to not only providing new method and clue for the prevention and treatment of pouchitis, but also offering a clue to the pathogenesis study of crohn disease and ulcerative colitis. So far, the relationship between the intestinal flora imbalance and the pathogenesis of pouchitis is not clear yet. We establish pouchitis rat model, the expected intestinal inflammatory disease model to dynamically test the changes of

*Corresponding author: Jian Zuo, Deputy Chief Technician, State Key Laboratory of Infectious Disease Diagnosis and Treatment, First Affiliated Hospital of Medical School of Zhejiang University, Hangzhou, Zhejiang Province, 310003, China.
Tel: +86 13805708352
E-mail: zuojian4743@163.com

Peer review under responsibility of Hainan Medical College.
Foundation project: It was supported by Natural Science Foundation of China (Grant Number: 81550049).

# These authors contribute equally to this work.

intestinal flora through high-throughput sequencing and the Real-time PCR quantitative techniques to discuss its mechanism of action in the intestinal inflammatory reaction, and provide a clue of the pathogenesis and treatment direction of the intestinal inflammatory disease.

2. Materials and methods

2.1. Materials

QIAamp DNA Mini Kit and Real-time PCR were purchased from Qiagen Co., Ltd.; the extraction kits of bacterial genome DNA were purchased from Tiangen BioTech Co., Ltd. A total of 70 SPF grade male Lewis rats with body weight of 300–325 g were purchased from Animal Experiment Center of Shanghai Jiao Tong University. This experiment was approved by Ethics Committee of Jinling hospital, and all operations were strictly abided by the specifications of Regulation of Experimental Animals. The experimental operations were finished at Department of Comparative Medicine, Jinling Hospital, and the operation of gene detection was finished at State Key Laboratory of infectious disease diagnosis and treatment, First Affiliated Hospital of Medical School of Zhejiang University.

2.2. Methods

2.2.1. Establishment of experimental ileoanal pouch rat model

A total of 50 Lewis male rats with body weight of 300–325 g were fed in a simulation environment with constant temperature. The rats were fed with standard rodent's foodstuff in the metabolism cages of 5–7 d in order to adapt to the cages before the operation. They were fasted before 24–36 h of the operation, and their drinking water was replaced with 5% glucose solution to avoid the delay of the healing of anastomotic stoma caused by high catabolism state. The rats were anesthetized with intramuscular injection without using antibiotics before the operation, then the intestinum crassum was resected after opening operation, and ileoanal pouch was established to coincide with rectum, then the abdominal cavity was closed. The operations were all conducted in the aseptic conditions. The rats were put on the water jacket heating pad 24 h after the operation, then they were put into metabolism cages after 24 h for 4 d and fed with 5% glucose solution only; they were fed with rodent's foodstuff soaked with glucose at the 6th day of the operation, and fed with standard rodent's foodstuff after they had tolerance of the food soaked with glucose. A total of 20 SD normal male rats were selected as control group, and their general conditions were observed and recorded, and all rats were executed at the 31st of the experiment and the mucous membrane samples were collected. The faeces samples were taken out for detection every week, and the samples were all preserved in the temperature of −80 °C.

2.2.2. Extraction of DNA of mucous membrane and total bacteria in faeces

The cells were mechanical cracked by glass bead breaker, and DNA of mucous membrane samples and the faeces of rats were extracted referenced to CTAB method; the QIAamp DNA Mini Kit (Qiagen) was used to extract the DNA of bacteria in mucous membrane and the QIAamp DNA stool Mini Kit (Qiagen) was used to extract the DNA of the faeces. All operations were strictly abided by the product specifications, and all the DNA samples were preserved in the temperature of −20 °C.

2.2.3. 16S rRNA PCR analysis and 454 high-throughput sequencing of intestinal bacteria

The DNA of total bacteria in faeces samples was used as model for the PCR analysis on V6–V8 fragment of 16S rRNA gene sequences of the bacteria, and the primers of PCR were U968-GC and L1401 with GC clips. The DNA of total bacteria in mucous membrane samples and faeces samples was extracted as the model for the PCR analysis on V3–V5 fragment of 16S rRNA gene sequences of the bacteria, the 16S rRNA fragment V3–V5 variable region, which is the amplification substance of PCR, was sequenced by Sangon Biotech (Shanghai) Co., Ltd. through 454 high throughput sequencing method. The sequences after the 454 sequencing were effectively screened, and the screening criteria showed as follow: 1) at least one fragment of the sequence was complete matching with barcode sequence and the primer sequence; 2) the length of the sequence was over 50 bp; 3) the number of blurry bases of the sequence was not over 2.

2.2.4. Quantitative analysis of samples by real-time PCR instrument

The total bacteria, *Firmicutes*, *Bacteroidetes*, IV *Clostridium coccoides* (*C. coccoides*) group, XIV *Clostridium leptum* (*C. leptum*) subgroup, *Bifidobacterium* and *Lactobacillus* in the mucous membrane samples and faeces samples were quantitatively analyzed by ABI 7500 real-time PCR instrument. The total reaction system of PCR was 20.0 μL: 10.5 μLof SYBR Green Supermix, 0.3 mol/L of upstream primers and downstream primers, 1.0 μL of DNA sample and 7.8 μL of aquae sterilisata. The represented nucleic acid of *Escherichia coli*, *Bacteroides thetaiotaomicron*, *C. coccoides*, *C. leptum*, *Faecalibacterium prausnitzii* (*F. prausnitzii*), *Lactobacillus sobrius* and *Bifidobacterium longum* were respectively used as model to amplify their 16S rRNA gene, and make corresponding quantitative standard curve. The density of DNA of the PCR production was detected after the purification of the production, and calculated its copy numbers according to the formula: Copy = (C/X) × 0.912 × 1012. The using primers and the reactions of real-time PCR were showed in Table 1.

2.2.5. Data analysis and processing

After the experimental data were preliminary statistically processed by Excel 2007, and the SPSS16.0 software was used for the significant analysis between pouchitis group and ileoanal pouch group through the one-way analysis of variance method. Chi-square test and Logistic regression were used to analyze the change over time of structure and number of intestinal flora, respectively.

3. Results

3.1. General conditions and pathological changes of pouchitis

There was significant weight loss, fecal occult blood positive and diarrhea in 30 rats at the 21st day of the experiment, which means the model was established successfully according to the