Establishment and evaluation of specific antibiotic-induced Inflammatory bowel disease (IBD) model in rats

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Abstract

Background: Physical and chemical methods have been established for rat enteritis model, but antibiotic induction has been relatively rare. This article aims to establish and evaluate rat model of Inflammatory bowel disease (IBD) using antibiotics

Methods: Eighty four female SD rats were divided into A-G group according to the dosage and method of antibiotics, among which group A was the control group and others were treated groups. The drug was stopped on the 7th day, the modeling period was 1-7 days, and the recovery period was 8-15 days. Half of the animals were dissected on 11th day and the other animals were dissected on 15th day. Record the food and water intake, body weight, and fecal weight for 2 hours on different days. Number of defecation of each rat was counted for analyzing diarrhea. Nine intestinal flora were analyzed by bacterial culture and three strains were analyzed by quantitative PCR. TNF-α, IL1-β, IL-6 and CRP in abdominal aorta blood were detected and analyzed. Colon and rectal tissues were pathologically examined for inflammation and scored.

Results: Rat weight, food intake, water intake, and two-hour feces were significantly different (P = .04, .016, <. 001, .009). Compared with group A, there were significant differences in 9 kinds of flora in the experimental group (all P <.001). Significant diarrhea existed in B-G rats dependent on drug (all P<.001). Bacteroides, Faecalibacterium prausnitzii, and Dialister invisus concentrations were analyzed by Quantitative real time polymerase chain reaction (q-PCR) and showed significant differences in groups A, C, and F (p = .033). There were significant differences about TNF-α, IL1-β, IL-6 and CRP between the groups (all P<.001). The colonic and rectal pathological inflammation scores of other groups were significantly different from those of the control group (all P <.001).

Conclusion: Specific antibiotic-induced enteritis model in SD rats is feasible.

1. Introduction

Inflammatory bowel disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC), which are common intestinal diseases of patients, and they are increasing year by year.[1] It has a higher incidence in Canada and reports of more difficult treatments.[2–4] Clinically, this type of patients has a slow treatment effect and a long treatment period, which have attracted the attention of gastrointestinal physicians and need expensive costs[4]. The disease is considered a patient's immune system disorder, and changes in the intestinal flora and its metabolites usually affect the patient's immune system, thereby inducing the disease.[5, 6] Incorporating a combination of serological, genetic, and inflammation markers improved the accuracy of identifying IBD [7]. Because of the refractory nature of the disease, many countries have increased their financial investment in research into the disease and have achieved significant results[8, 9]. With the changes in the dietary structure of Chinese residents, the incidence of IBD has also increased, and many scientific research institutions in China have increased their investment in this disease. IBD patients usually have intestinal flora disturbances[10], and it is unclear whether various causes of flora disturbances cause IBD.

In order to have a deeper understanding of the disease, many scholars use animal models to study the disease.[11] Rats are good mammals for research.[12] Sprague Dawley (SD) rats are easy to raise and are easily controlled due to their mild temperament. They have been used for the establishment of various disease models. At present, rat enteritis models mostly use physical and chemical factors.[13, 14] However, these methods easily lead to intestinal perforation and intestinal necrosis in rats, which can lead to failure of modeling. New modeling methods need to be studied and discussed. Because intestinal flora imbalance may cause enteritis, we thought of using flora imbalance for modeling. Antibiotics often cause imbalances in the intestinal flora.

Therefore, in this article, we used different doses of clindamycin (single use) and different doses of clindamycin plus ampicillin and streptomycin (combination) to cause intestinal flora disturbance in rats. The inflammatory factors in the abdominal aorta and the inflammation of the colon and rectum were analyzed in order to assess whether modeling is feasible.

2. Materials And Methods

Rats

Sprague Dawley (SD) female rats (n = 84), obtained from Liaoning Changsheng Biotechnology Co., Ltd. ranged in size from 172.4 to 179.5 g. All animals were introduced to quarantine and adaptive feeding in the Specific Pathogen Free (SPF) barrier system of this institution for 9 days. All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80 − 23) revised in 1996. All experimental procedures and animal handing were performed in accordance with the guidelines of the International Association for the Study of Pain and the animal protocols were approved by Xi'an United Nations Quality Detection Technology CO.,Ltd Animal Committee. The authors tried all efforts to minimize the number of animals used.

Experimental drugs, reagents, and instruments

The used drugs were used in our experiments: clindamycin hydrochloride(Shanghai Maclean Biotechnology Co., Ltd.) and 99% purity, ampicillin (Hebei Bailingwei Superfine Material Co., Ltd.) and streptomycin (Tianjin Guangxia Fine Chemicals Institute) with ≥ 90% purity.

We also used rat IL-1β, rat IL-6, rat TNF-α, and rat CRP reagents from Bioswamp (Wuhan, China). We purchased soil genomic DNA rapid extraction kit, rapid competent cell preparation kit (one-step method), SanPrep column plasmid DNA small amount extraction kit, SanPrep column DNA gel recovery kit from Sangon Biotech (Shanghai),purchased Taq Plus DNA Polymerase, Agarose B, 4S Red Plus Nucleic Acid Stain (10,000X Aqueous Solution) from BBI, and GeneRuler DNA Ladder Mix (from Thermo Scientific), pMD® 18-T Vector (from TAKARA Bio Ink)

Bacteroides-Bile-Enterprise Agar (BBE), Lactobacillus selective agar,Anaerobic bacteria agar ,TPY agar medium, mannitol sodium chloride agar medium, Eosin Methylene Blue agar (EMB) ,CATC agar ,Reinforced Clostridium Culture Medium were purchased and utilized for bacterial culture.

The electric day constant temperature incubator was purchased from Tianjin Taisite Instrument Co., Ltd. (Tianjin City, China). The electric day constant temperature incubator was purchased from Shanghai Yiheng Scientific Instrument Co., Ltd. (Shanghai, China). The Lab systems Multiskan MS micro plate reader was purchased from Thermo Fisher (Pittsburgh, PA, USA). The low-speed condensation centrifuge, was purchased from Shanghai Luxiangyi Centrifuge Instrument Co., Ltd. (Shanghai, China). The electronic balance was purchased from Yuyao Jinnuo Tianping Instrument Co., Ltd. (Zhejiang Sheng, China). The electronic balance was purchased from Sedolis Instrument Co., Ltd. (China). The upright microscope was purchased from Japan Nikon Guangxuan Microscope Manufacturing Co., Ltd. (Tokyo, Japan).

In order to do real-time quantitative PCR, we used the following instruments: clean bench (purchased Jiangsu Su Clean Chemical Equipment Factory), high-speed refrigerated centrifuge (Anhui Zhongke Zhongjia Instrument Co., Ltd.), electrophoresis instrument (Beijing Liuyi), electrophoresis tank (Shanghai Jingyi Plexiglass Products Instrument Factory), Gel Imaging System (Shanghai Furi Technology Co., Ltd.), Micro-Spectrophotometer (Merinton Instrument, Inc), PCR Reaction Amplifier (BIO), Pipette (range 100–1000 ul, 20-200ul, 0.5-10ul) (BBI, Canada), sequencer (ABI, Foster, CA, USA), StepOne fluorescence quantitative PCR instrument (ABI, Foster, CA, USA).

Animal grouping and modeling

Group A was the control group, group B was the low-dose clindamycin group (250 mg/kg), group C was the middle-dose clindamycin group (500 mg/kg), and group D was the high-dose clindamycin group (750 mg/kg). Group E was the low-dose triple antibiotic group (clindamycin, ampicillin, and streptomycin; 250 mg/kg, 272.1 mg/kg, and 136.1 mg/kg, respectively). Group F was the medium-dose triple antibiotic group (clindamycin, ampicillin, and streptomycin; 500 mg/kg, 563.7 mg/kg, and 281.8 mg/kg, respectively). Group G was the high-dose triple antibiotic group (clindamycin, ampicillin, and streptomycin; 750 mg/kg, 835.8 mg/kg, and 417.9 mg/kg, respectively).Each group included 12 rats.The experiment was divided into two

stages: the modeling period (days 1–7) and the recovery period (days 8–15). The administration volume was 10 ml/kg once a day through stomach feeding by oral needle during the modeling period between 8:30 – 10:00 AM. The intragastric administration was stopped at 8th day. The weight, food-intake volume, water-intake volume, and stool samples were taken on days 1, 3, 5, 7, 9, 11, and 14 within 2 hours were collected. For each rat, the fecal microbial flora on the 1st, 4th, 8th, 11th, and 14th days were examined. On day 11 and day 15, half of each animal was dissected with 2% pentobarbital sodium (0.2 ml/100 g) by intraperitoneal anesthesia injection. The rats were euthanized by draining abdominal aortic blood under anesthesia, and then colon and rectal tissues were taken. (Fig. 1A-C)

Baterial Culture

The included nine microbial organisms detected were Staphylococcus aureus, Bifidobacterium, yeast, Bacteroides, Clostridium, anaerobic bacteria, E. coli, Enterococcus, and Lactobacillus.

We Mixed 1 g of feces with 9 ml of tryptone soy broth, diluted to the appropriate concentration, and took 20 ul of the sample and spread it evenly on the agar medium using a coating bar. Organisms were plated onto mannitol sodium chloride agar medium plates, EMB, and CATC agar plates under aerobic conditions at 37 ^{o}C for 48 hours. Organisms cultured on TPY agar medium plates, BBE agar plates, reinforced Clostridium medium plates, anaerobic agar plates, and lactobacillus selective agar plates were cultured at 37 ^{o}C for 48 hours in anaerobic conditions. Organisms inoculated on DRBC agar plates were cultured for 5 days at 28 ^{o}C in aerobic conditions. Colonies were enumerated using the following formula: number of colonies (cfu/g) = number of plate colonies × 50 × dilution factor, with x 10^{6} (E6) as a uniform unit.

Diarrhea within 2hours after administration

Within 2 hours after drug administration, the number of defecation of each rat in each group was counted to determine if they had diarrhea and how severe it was. At the same time, the changes of diet and water consumption of rats were observed. We found that the rats had no obvious dehydration and could tolerate the experiment.

Real-time quantitative PCR analysis

Soil genomic DNA rapid extraction kit (B518233, Shengong Biological Co., Ltd., Shanghai) was used to extract fetal DNA from SD rats. The process is as follows:1). Weigh 400 mg of SD rat feces, add 400 μl of 65 ° C pre-warmed Buffer SCL, mix by shaking, and place in 65 ° C water bath for 5 min.2). Centrifuge at 12,000 rpm for 3 minutes at room temperature. Pipette 350 μl of the supernatant into a clean 1.5 ml centrifuge tube.3). Add equal volume of Buffer SP, mix by inversion, and ice bath for 10 min.4). Centrifuge at 12,000 rpm for 3 minutes at room temperature. Pipette 350 μl of the supernatant into a clean 1.5 ml centrifuge tube.5). Add 200 μl of chloroform, mix well, and centrifuge at 12,000 rpm for 5 minutes. Pipette the upper aqueous phase into a clean 1.5 ml centrifuge tube.6). Add 2 volumes of absolute ethanol, invert 8 times to mix thoroughly, and leave at room temperature for 3 min. Centrifuge at 10,000 rpm for 5 min at room temperature and discard the supernatant.7). Add 1 ml of 75% ethanol, rinse by inversion for 3 minutes, centrifuge at 10,000 rpm for 2 minutes, and discard the supernatant.8). Repeat step 7 once.9). Open the lid and invert for 10 minutes at room temperature until the residual ethanol is completely evaporated.10). Dissolve the resulting DNA in 70 μl TE Buffer. The extracted DNA can be immediately used for further experiments. qPCR experiment was performed by pMD™18-T Vector Cloning Kit(TAKARA BIO INC, Dalian,China) ..Bacteroides, Faecalibacterium prausnitzii, Dialister invisus in rats fecal were collected and analyzed by PCR. After PCR, electrophoresis analysis was performed two times for the three organisms using 1.5% agarose syrup electrophoresis map.

Analysis of inflammatory factors

On the 11th and 15th days, half of the rats were dissected in each group with 2% pentobarbital sodium (0.2 ml/100 g) by intraperitoneal anesthesia injection, and the abdomen was cut, and 5 mL blood was drawn from the abdominal aorta into the

blood collection tube). TNF-α, IL-1β, IL-6, and CRP was detected in the blood serum without diluting by an enzyme-linked immune sorbent assay (ELISA).

Colon and rectal pathological inflammation assessment

0 points: no inflammation; 1 point: a small amount of multifocal neutrophil infiltration (< 10 per HPF); 2: a moderate multifocal neutrophil infiltration (more submucosal involvement) (10 ~ 50 / HPF); 3 points: a large number of multifocal and even aggregated neutrophils infiltration (more submucosa involvement and muscle layer) (> 50 / HPF); 4 points: the lesions involved the same 3 points, but abscesses or more extensive muscle layer involvement occurred.

1 + 2 + 3 Score < 3 is grade I: mild; 1 + 2 + 3 Score is 4 to 6 is grade II: moderate; 1 + 2 + 3 A score of 7 to 9 is grade III: severe; 1 + 2 + 3 A score of > 10 is grade IV: extremely severe.

Ethics

This study follows the Basel Declaration 2010 and Institutional Animal Care and Use Committee (IACUC) of Xi'an United Nations Quality Detection Technology CO.,Ltd. We use animals to a minimum in terms of animal welfare principles and without affecting the accuracy of the experiment. All applicable international ,national and/or institutional guidelines for care and use of animals were followed.

Statistical analysis

Primer Premier 5.0 software was used for sequencing primer design. SPSS 21 software (IL, USA)was utilized to analyze all data. Measured data were analyzed by ANOVA and F-test. Two groups within the group were compared using the LSD method. Crosstabs were also used for measurement data analysis. Categorical variables were used by crosstabs and chi-square test. The independent sample T-test was used for comparative analysis between two sets of measured variables.

3. Results

Comparison of basic indices

The average starting weight of all rats was 17.26 ± 2.49 g, and there was no significant difference in weight between groups A-G ($P < .05$). There were significant differences from groups A (control) to G (treated) in weight, food intake, water intake, and stool 2 hours post antibiotic use ($P = .04$, .016, < .01, and .009, respectively). Means and standard deviations are shown in Table 1 and Fig. 2(A-D).

Table 1

Comparison of basic status for rats at total days (g,`X ± S)

	Weight	Food intake	Water intake	Stool in 2 h
A	204.02 ± 13.5	19.02 ± 3.63	38.86 ± 4.73	0.96 ± 0.48
B	203.31 ± 17.2	19.58 ± 2.83	49.76 ± 11.31	2.20 ± 1.38
C	208.09 ± 22.15	18.93 ± 2.75	44.40 ± 9.24	2.23 ± 1.09
D	188.13 ± 24.05	15.47 ± 5.66	41.96 ± 18.95	2.47 ± 1.53
E	209.00 ± 18.58	18.80 ± 2.11	60.33 ± 9.02	2.82 ± 2.01
F	210.70 ± 19.18	20.31 ± 3.57	52.68 ± 10.23	3.09 ± 1.79
G	212.12 ± 20.31	20.66 ± 3.13	53.47 ± 7.84	2.33 ± 1.55
F	2.313	2.807	6.213	3.043
P	0.04*	0.016*	< 0.001**	0.009*
*P < 0.05,**P < 0.001				

Comparison of nine organisms of bacterial between groups

Staphylococcus aureus, Bifidobacterium, yeast, Bacteroides, Clostridium, anaerobic bacteria, E. coli, Enterococcus, Lactobacillus were cultured and counted using special medium.(Fig. 3). All collected bacterial loads for these 9 species according to above methods were counted and compared between A group to B-G groups. The unit of bacteria in the stool is (CFU / g).The details were as followed: (A): 1376.7 ± 3683.8(95% CI: 562.15-2191.26); (B): 687.06 ± 1498.74 (95% CI: 355.65-1018.45); (C): 1474.89 ± 4187.53 (95% CI: 548.96-2400.83); (D): 478.17 ± 1758.11 (95% CI: 65.03-891.31); (E): 664.50 ± 1567.91（95% CI: 317.80-1011.19); (F): 403.77 ± 1171.99 (95% CI: 144.62-662.92); (G): 3609.76 ± 21206.52 (95% CI: -1079.38 ± 8298.91).The compared results were all < .001(Table 2). Figure 4 (A-E) showed the comparison results of the nine organisms of bacteria in each group on days 1, 4, 8, 11, and 14 (all P < .001).

Table 2

Comparison of total nine organisms of bacteria between A and B-G groups (E6)

		A	B	C	D	E	F	G
X ± S		1376.7 ± 3683.8	687.1 ± 1498.7	1474.9± 4187.6	478.2 ± 1758.1	664.5 ± 1567.9	403.8 ± 1172.0	3609.8 ± 21206.5
95%CI for mean	lower	562.1	355.7	549.0	65.0	317.8	144.6	-1079.4
	upper	2191.3	1018.5	2400.8	891.3	1011.2	662.9	8298.9
P			< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**	< 0.001**
**P < 0.001								

Table 3 Comparison of defecation numbers between A group and B to G group within 2h after administration in all included days(`X±S)

groups	N	Diarrhea	P
A	12	0.833±0.578	
B	12	2.583±0.900	<0.001**
C	12	4.250±0.754	<0.001**
D	12	4.667±0.492	<0.001**
E	12	5.083±0.669	<0.001**
F	12	5.250±0.622	<0.001**
G	12	6.500±1.087	<0.001**

**P<0.001

Diarrhea analysis

Table 3 revealed the number of defecation within 2hours after drug administration for each rat in each group in all included days. We considered if the number were more than or mean to 2, then diarrhea existed. Our research showed B-G group had diarrhea dependent on drug dose and methods which indicated that all groups (B-G) had significance by comparison with A group (all P < .001).Within group comparison, C/D had no significance (P = .179)while C/E and C/F had significance (P = .008,P = .002 respectively) as shown in Fig. 4 (F).

Real-time quantitative PCR analysis of three strains

Bacteroides forward-primer (5'-3') is TTAAGTATTCCACCTGGGGAGT and reverse-primer(5'-3') is TTAAGCCCGGGTAAGGTTCCT with product size of 156 bp. Faecalibacterium prausnitzii forward-primer (5'-3') is CACGGCTCTGGAAATCTATGT and reverse-primer(5'-3') is GCACAATGAGCATACCGAGTT with product size of 140 bp. Dialister invisus forward-primer (5'-3') is AGACGGAAACGACTGCTAATACC and reverse-primer(5'-3') is

CAGCTAATCAGACGCAAACCC with product size of 116 bp. The three strain gene fragments were retrieved from the NCBI GenBank database. (listed in Table 4). Figure 5 (A-C) showed amplification plot of the three strains, and the agarose syrup electrophoresis picture is shown in Fig. 5 (D).OD and concentration of the three strains were compared between A,C,F groups showing there were no significant difference about OD (p = .550),while significant difference existed about concentration (p = .033).Mean and standard deviations were listed in Table 5.

Table 4
Sequences of primers used for real-time quantitative PCR

Target bateria	**Forward(5'-3')**	**Reverse(5'-3')**	**Product size(bp)**	**Accession number**
Bacteroides	TTAAGTATTCCACCTGGGGAGT	TTAAGCCCGGGTAAGGTTCCT	156	CR626927
Faecalibacterium prausnitzii	CACGGCTCTGGAAATCTATGT	GCACAATGAGCATACCGAGTT	140	NZ_PXUP01000071
Dialister invisus	AGACGGAAACGACTGCTAATACC	CAGCTAATCAGACGCAAACCC	116	LT223661

Table 5

Comparison of Bacteroides, Faecalibacterium prausnitzii, Dialister invisus examination by Reartime PCR between A,C, F groups (ng/ul), (ˉX ± SD)

	A group	C group	F group	F Value	P Value
Realtime PCR	21.72 ± 10.60	34.60 ± 19.85	26.14 ± 19.86	10.711	0.033*
OD	1.88 ± 0.18	1.79 ± 0.25	1.79 ± 0.16	0.612	0.550

Changes of abdominal aortic inflammatory factors

Mean, standard deviation and 95%CI(Confidence Interval) of TNF-α, IL1-β, IL-6, CRP were counted and compared between A to G. showing all existed significant difference(all $P < 0.001$).The values were shown in Table 6 and Fig. 6(A-D).

Table 6

Comparison of inflammation factors from group A to group G (pg/ml)

		N	Mean	SD	95% Confidence Interval for Mean		F	P
					Lower Bound	Upper Bound		
TNF	A	12	179.1	25.9	162.7	195.8	204.89	< 0.001**
	B	12	207.0	12.0	199.4	214.6		
	C	12	244.1	10.8	237.2	251.0		
	D	12	272.3	18.3	260.7	283.9		
	E	12	276.0	8.3	270.8	281.3		
	F	12	311.8	9.0	306.0	317.9		
	G	12	361.4	12.2	353.6	369.2		
IL1β	A	12	65.8	19.2	53.5	78.0	341.57	< 0.001**
	B	12	100.3	8.4	94.9	105.6		
	C	12	130.6	12.5	122.7	138.6		
	D	12	178.2	11.4	171.0	185.4		
	E	12	218.8	36.5	195.7	242.1		
	F	12	308.2	12.6	300.2	316.2		
	G	12	345.8	22.3	331.7	360.0		
IL6	A	12	124.0	24.8	108.2	139.8	354.15	< 0.001**
	B	12	172.2	19.0	160.1	184.3		
	C	12	222.0	27.9	204.2	239.7		
	D	12	276.0	16.8	265.3	286.6		
	E	12	308.7	7.3	304.0	313.3		
	F	12	352.0	11.2	344.8	359.1		
	G	12	414.2	15.5	404.4	424.1		
CRP	A	12	9.8	2.8	8.0	11.6	1153.5	< 0.001**
	B	12	15.9	2.2	14.5	17.3		
	C	12	25.7	2.7	24.0	27.4		
	D	12	35.9	1.8	34.7	36.9		
	E	12	48.7	1.9	47.5	49.8		
	F	12	57.2	1.6	56.2	58.2		
	G	12	71.2	2.8	69.4	72.92		

*p < 0.05indicate significant difference,**P < 0.001.

Comparison of pathological inflammation scores

Colonic and rectal tissues were scored and compared for pathological inflammation according to the methods described above after H&E staining. For colon, inflammation scores of A,B,C,D,E,F,G were 0, 2.5 ± 0.24, 3.5 ± 0.61, 8 ± 0.26, 5.5 ± 0.27, 9.5 ± 0.22 and 10.5 ± 0.35 respectively. Results of comparison with A group showed significant difference(all P < .001).For rectum, inflammation scores of A,B,C,D,E,F,G were 0, 3 ± 0.27, 5 ± 0.15, 10 ± 0.13, 6 ± 0.32, 10 ± 0.48, 10 ± 0.59 and significance difference existed compared with A group.(all P < .001), Table 7 listed the details. The line chart shows a significant increase in colonic and rectal pathological inflammation scores. However, there was no significant difference in the degree of inflammation between the two tissues (P = .710).(see Fig. 5(E)). Figure 7 and Fig. 8 showed the colon and rectal pathological slice pictures of the rats (* 100) between A to G group which indicated inflammation scores were all significant higher in rats dissected in 11th day than those in 15th day about colon tissue and rectum tissue (data were not shown in this paper).

Table 7
Mean inflammation score by pathology (ˉX ± S)

groups	Colon	P	Rectum	P
A	reference		reference	
B	2.5 ± 0.24	0.007	3 ± 0.27	0.004
C	3.5 ± 0.61	0.001	5 ± 0.15	< 0.001
D	8 ± 0.26	< 0.001	10 ± 0.13	< 0.001
E	5.5 ± 0.27	< 0.001	6 ± 0.32	< 0.001
F	9.5 ± 0.22	< 0.001	10 ± 0.48	< 0.001
G	10.5 ± 0.35	< 0.001	10 ± 0.59	< 0.001

4. Discussion

This article has made an important discussion on antibiotic-induced rat IBD model. It is important to choose the appropriate animal model of IBD to evaluate the non-clinical anti-inflammatory effects of the drug. Some scholars have conducted comparative research on rat IBD models caused by different chemical factors, and believe that each has its own advantages and disadvantages.[13] A highly reversible and reliable IBD rat model has broad application prospects for new drug treatment of IBD. Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders that affect individuals throughout life. Although the etiology and pathogenesis of IBD are largely unknown, studies with animal models of colitis indicate that dysregulation of host/microbial interactions are requisite for the development of IBD.[15]

The modeling method of this article is from the 2015 master's thesis of Wendi Zhang of Southern Medical University.[16] In her paper, he compared and analyzed the imbalance of rat enteritis flora induced by antibiotics. Although we used her modeling method, the research focuses are different. We focused on the changes of 9 kinds of flora and the changes of inflammatory factors in the blood. At the same time, we compared and analyzed the colon and rectal pathological inflammation in the two stages of model recovery period.

In our paper, we analyzed the changes of 9 intestinal flora during the modeling period (days 1 and 4) and the recovery period (days 8, 11 and 14). Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections.[17, 18] we noticed that as the dose of the antibiotic increased, the increase in bacterial load was not obvious, indicating that the drug inhibited the strain. Bifidobacterium are defined as a group of living microorganism supplements that confer health benefits on the host when administered in adequate amounts.[19] The beneficial bacterium was significantly reduced in B, D,E,F group relative to the control group. Yeast cells are often employed in industrial fermentation processes for their ability to efficiently convert relatively high concentrations of sugars into ethanol and carbon dioxide.[20] Yeast is not a common intestinal bacterium, and it is absent in the control group. It was only found in the low- and middle-doses of the triple-agent groups. We

considered it was the results of intestinal flora disorders. Bacteroide is a gram-negative, non-spore, obligate anaerobic bacillus. Our study showed a significant reduction in the high-dose medication group, indicating that it was sensitive to high-dose and combination drugs and it was relative with type 2 diabetes.[21] Clostridium organism are anaerobic, Gram-positive, rod-shaped, endospore-forming bacteria belonging to the phylum Firmicutes, and they constitute both a class and a genus in the phylum.[22] Clostridium decreased in the single-agent group and the combined drug group increased. Anaerobic bacteria have pivotal roles in the microbiota of humans and they are significant infectious agents involved in many pathological processes.[23] Pathogenic variants of E. coli (pathovars or pathotypes) cause much morbidity and mortality worldwide.[24] Enterococcus strains that adhere strongly to the intestinal epithelium, form biofilms and possess antioxidant defence mechanisms seem to have the greatest influence on the inflammatory process.[25] The genus Lactobacillus consists of 173 species and many genomes are available to study taxonomy and evolutionary events.[26] In another article, we elaborate on the changes of these nine floras.In general, with the increasing dose of antibiotics and the enhanced degree of combination, the beneficial bacteria decreased significantly, while the pathogenic bacteria increased significantly.

Subsequently we performed real-time quantitative PCR analysis on three strains of Bacteroides, Faecalibacterium prausnitzii, and Dialister invisus (control group, medium-dose alone group, and medium-dose combined group). The results showed that the lower the number of copies, the higher the dose and the combination. Bacteroides organism makes up a significant fraction of the human gut microbiome, and can be probiotic and pathogenic, depending upon various genetic and environmental factors. These can cause disease conditions such as intra-abdominal sepsis, appendicitis, bacteremia, endocarditis, pericarditis, skin infections, brain abscesses and meningitis.[27–29] There is an increasing interest in Faecalibacterium prausnitzii, one of the most abundant bacterial organisms found in the gut. F.prausnitzii phylogroups can be found within this species and their distribution is different between healthy subjects and patients with gut disorders. It also remains unknown whether or not there are other phylogroups within this organisms, and also if other Faecalibacterium species exist.[30, 31] Dialister invisus is reported to be low or not expressed in IBD patients.[32] Our research showed that Dialister invisus is little expressed in the model.

IBD often is associated with diarrhea[33, 34].The present study showed that diarrhea appeared in treated groups and the severity depended on the dose and method of administration. This potentially suggested the feasibility of antibiotic-induced IBD rat models.

Next our study found that TNF-α and IL-6 were significantly higher than the control group except for group D. However, there was no significant relationship with the dose administered and cytokine levels. TNF-α has been reported as a potent stimulator of IL-6 production.[35–37] Inflammation induces IL-1β production in Kupffer cells and hepatocytes.[38] In our study, IL-1β was significantly increased compared with the control group, showing elevated value dependent on dose administration. CRP is currently a hot spot for studying inflammation and related diseases.[39–41] Our results showed that elevated CRP was associated with those groups that received antibiotic compared with the control group.

Pathological examination is an important method to judge the degree of tissue inflammation. Colon and rectal tissues after H&E staining were scored based on the degree of neutrophil invasion. This study found that the degree of inflammation of the intestinal mucosa of rats dissected on day 11 was more severe than that of rats dissected on day 15, which may be related to the self-recovery of the intestinal flora. Deregulation of host-microbiota interactions in the gut is a pivotal characteristic of Crohn's disease. It remains unclear, however, whether commensals and/or the dysbiotic microbiota associated with pathology in humans are causally involved in Crohn's pathogenesis.[42] We investigated the degree of inflammation of colon and rectal tissues in antibiotic-induced SD rat enteritis models with drug doses and combined doses significantly increasing. And at the same time we found that the greater the degree of flora disturbance, the more severe the inflammation of the colon and rectal tissues, but there were no significant about inflammation score between colon and rectal tissue. This may be related to the release of inflammatory factors by the intestinal disorder flora.[43] It may also be related to the damage of intestinal mucosa caused by the release of metabolites by pathogenic bacteria to induce humoral and cellular immunity.[43, 44].

However, there are some shortcomings in this paper, such as not deep discussion about inflammation factors with intestinal mucosa necrosis. And the molecular mechanism of IBD caused by intestinal microbial disorders has not been explored in this study.

5. Conclusion

The use of specific antibiotics can cause imbalance in the intestinal flora ofrats and cause inflammatory changes in the intestinal mucosa. Specific antibiotic-induced IBD model in SD rats is feasible.

Declarations

Ethics approval and consent to participate

This study follows the Basel Declaration 2010.Most of the authors of this article have been trained in animal experiments and have obtained a certificate of competency. We use animals to a minimum in terms of animal welfare principles and without affecting the accuracy of the experiment. Xi'an United Nations Quality Detection Technology laboratory was commissioned to perform our experiments under his IACUC permission. All applicable international ,national and/or institutional guidelines for care and use of animals were followed.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author.

Competing interests

The contents of this manuscript have not been copyrighted or published previously. There are no directly related manuscripts or abstracts, published or unpublished, by any authors of this manuscript. The authors indicated no conflicts of interests.

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Authors' contribution

All authors discussed the methodology and considered it available. GJT ,HQ analyzed all experimental data. XFL, DLL, JL, JC reviewed the statistical results and participated in all Figures drawing and stitching with GJT. GJT wrote the paper. The research teams of Xi'an United Nations Quality Detection Technology Laboratory performed the experiment. All authors reviewed the manuscript.

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Figures

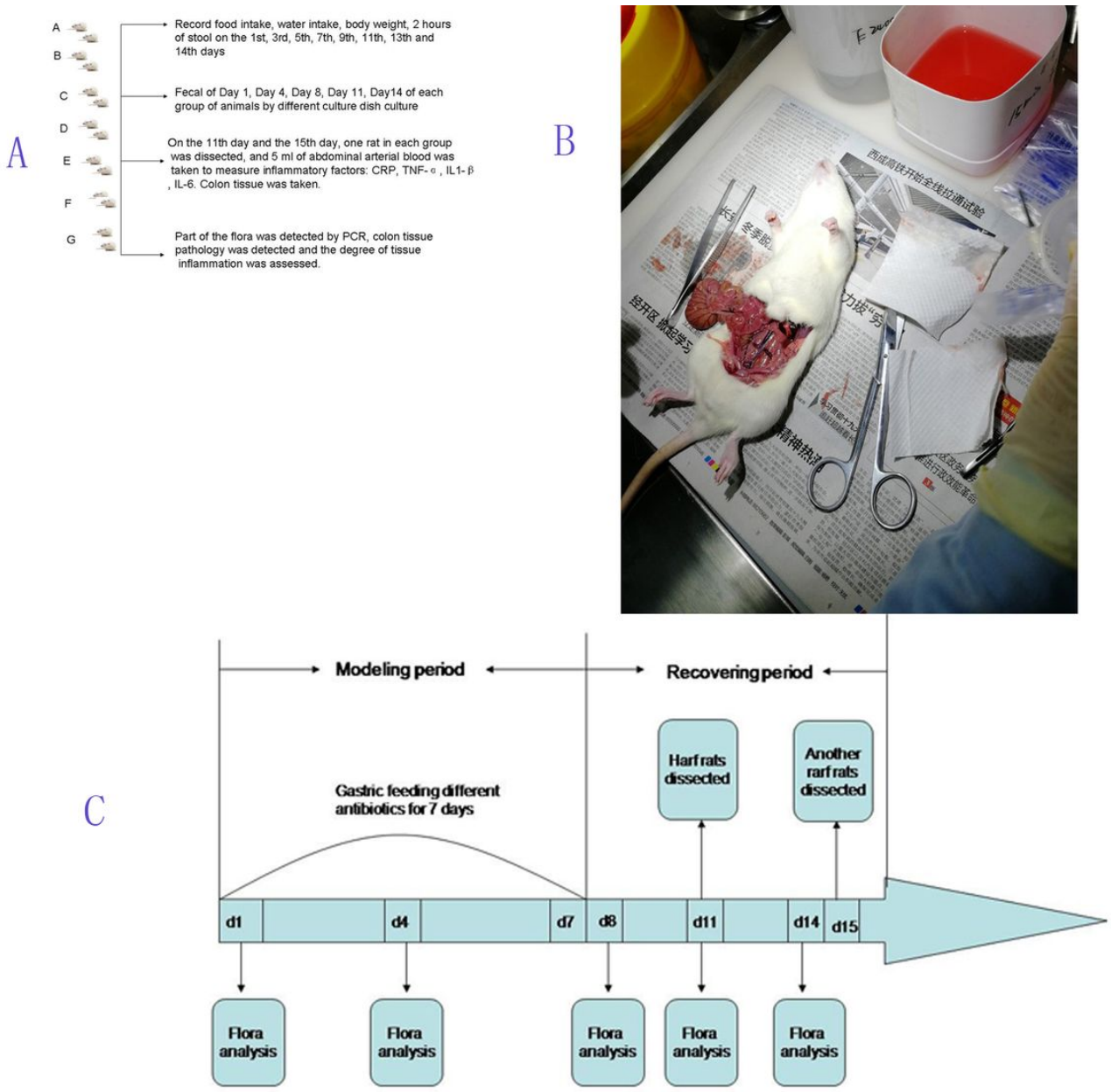

Figure 1

Study flow and method. A: Eight four SD rats of similar body weight were randomly divided into A-G 7 groups, 12 in each group, of which group A was the control group, B-D groups was the low-dose, middle-dose, and high-dose clindamycin administration group alone; E-G groups was a low-dose, middle-dose, high-dose clindamycin, ampicillin, and streptomycin combination administration group. B: SD rats were anesthetized with 2% pentobarbital sodium (0.2 ml / 100 g) by intraperitoneal injection on day 11 and day 15, and 5 ml of abdominal aortic blood was taken. Colon and rectal tissues were taken after being euthanized. C: The schematic diagram is the modeling process, 1-7 days is the model period for feeding specific antibiotic, and 8-15 is the recovery period after specific antibiotic withdrawal.

Figure 2

Basic indicators of the SD rat feeding process. A: High-low maps of animal weights comparison in each group on1st, 3rd, 5th, 7th, 9th, 11th, 13th, and 14th days. There were statistical differences between the groups, P = .04; B: Histograms of the food

intake of the animals in each group on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th, and 14th days. There was statistical difference between the groups, P <.016; C: Heat maps of animals in each group on1st, 3rd, 5th, 7th, 9th, 11th, 13th, and 14th days. There was a statistical difference between the groups, P <.001; D: Bar graphs of animals in each group for 2 hours on day 1, 3, 5, 7, 9, 9, 11, 13, 14 There was a statistical difference between the groups, P = .009.

Figure 3

A picture of counted bacteria specimen under a microscope on fourth day. a: Staphylococcus aureus; b: Bifidobacterium; c: yeast; d: Bacteroides e: Clostridium; f: anaerobic bacteria; g: E. Coli; h: enterococcus; i: Lactobacillus

Figure 4

Nine kinds of flora were compared in each group on days 1, 4, 8, 11, and 14, respectively. A: comparison on the first day; B: comparison on the 4th day; C: comparison on the 8th day; D: comparison on the 11th day; E: comparison on the 14th day, all P <.001. Because some data is 0, the standarddeviation cannot be added to the bar chart.

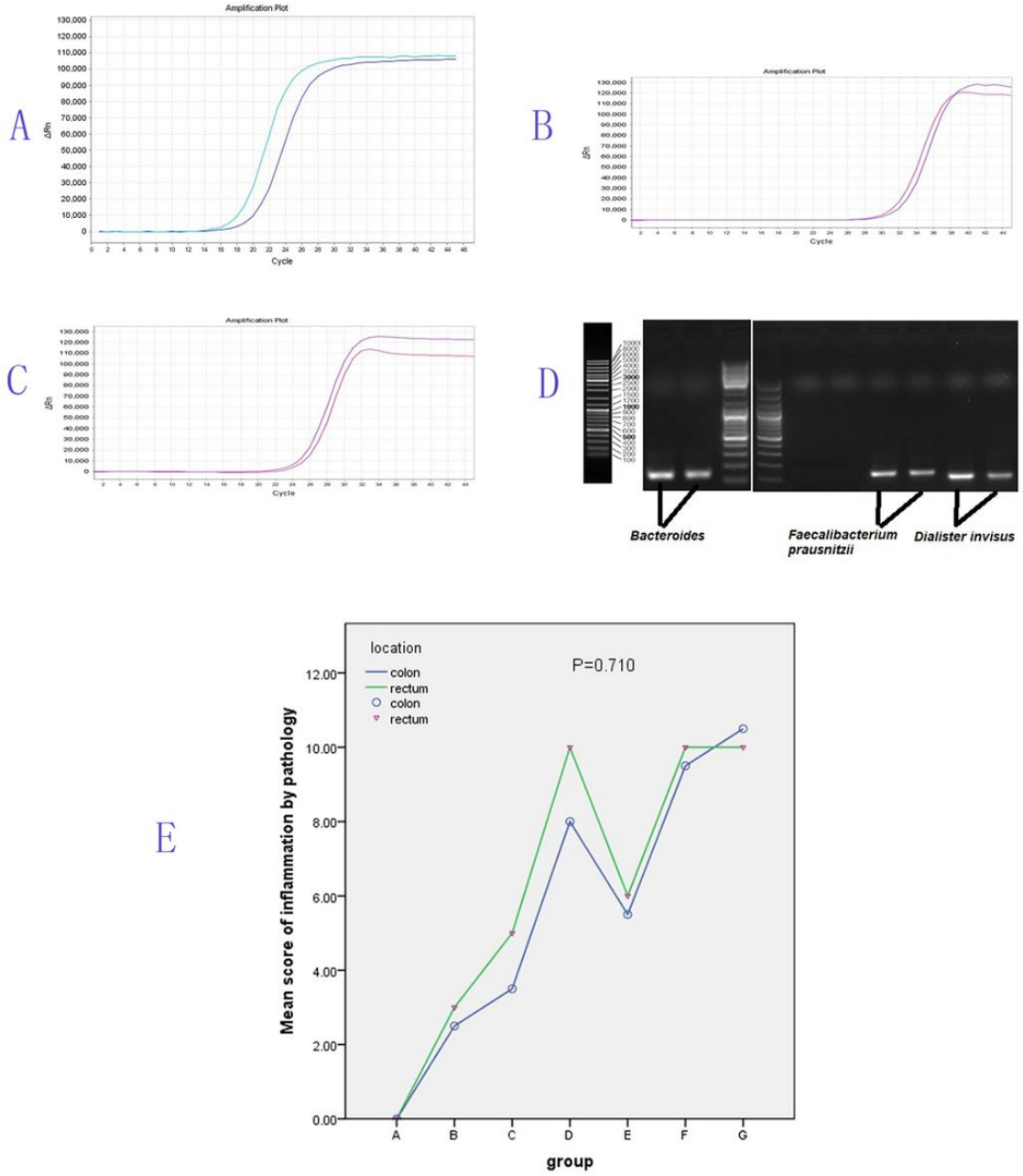

Figure 5

Real-time quantitative PCR doubling curve graph and agarose syrup electrophoresis graph of three strains, and line graph of inflammation score of colon and rectal tissue. A: Bacteroides amplification plot; B: Faecalibacterium prausnitzii amplification plot; C: Dialister invisus amplification plot; D: Bacteroides, Faecalibacterium prausnitzii, Dialister invisus agarose syrup electrophoresis picture, each done twice; E: Colon and rectal tissue inflammation score line chart, showing differences in each group, among which colon tissue inflammation score A / B: P = .007; A / C: P = .001; A / D, A / E, A / F, A / G: all P <.001;and rectum tissue inflammation score: A/B:P=.004, all others :all P<.001.But the score between colon and rectum are not significant, P=.710.

Figure 6

Changes of four inflammatory factors in each group. A: TNF-α comparison between groups, F=204.882,P<.001.LSD showed A/B,A/C,A/D,A/E,A/F,A/G, all P<.001;D/E ,P=.538 .B: IL1-β comparison between groups, F=341.573，P<.001 P<.001.LSD

showed all P<.001. C: IL-6 comparison between groups, F=345.145,P<.001, LSD showed all P<.001. ,D: CRP comparison between groups,F=1153.49,P<.001, LSD showed all P<.001.

Figure 7

Colonic HE staining pathological picture (* 100), the left is the dissection of the rat colon tissue on the 11th day, and the right is the dissection of the rat colon tissue on the 15th day. In group A, there was no obvious neutrophil infiltration and mucosal edema; in group B-G, neutrophil infiltration became more and more serious, mucosal edema became more and more obvious, and even mucosal necrosis and focal ulcer formed.

Figure 8

Rectal tissue HE staining pathological picture (* 100), the left side is the dissection of rat rectum tissue on the 11th day, and the right side is the dissection of rat recum tissue on the 15th day. In group A, there was no obvious neutrophil infiltration and mucosal edema; in group B-G, neutrophil infiltration became more and more serious, mucosal edema became more and more obvious, and even mucosal necrosis and focal ulcer formed.

Supplementary Files

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