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Diversity of intestinal microbiota and inflammatory cytokines after severe trauma

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Accumulating evidence has reported that the intestinal microbiota could play important roles in the occurrence and progression of severe trauma. However, the hypothesized potential targeted intestinal microbiota to mediate and regulate the levels of inflammatory cytokines and promote rapid recovery of body after severe trauma remains unclear. This study was aimed to explore the changes and correlation of intestinal microbiota and inflammatory cytokines in rats with severe crush and fracture trauma. The controlled laboratory study design was used, and a crush and fracture severe trauma rat model was established. 16S rRNA high-throughput gene sequencing and ELISA were used to analyze the changes in intestinal microbiota and inflammatory cytokines within one week after trauma. The correlation between intestinal microbiota and inflammatory cytokines was also analyzed. Loss of overall diversity and expansion of intestinal microbiota in the rats due to severe trauma was observed. Specifically, there was a significant increase in the abundance of *Muribaculaceae* [LDA (Linear Discriminant Analysis)-value = 4.814, *P* = 0.014] after severe trauma, while *Prevotella* (LDA-value = 5.235, *P* = 0.020) and *Alloprevotella* (LDA-value = 4.443, *P* = 0.015) were slightly lower in the trauma group than in the control group. The levels of inflammatory cytokines (IL-1α, IL-6, IL-8 and TNF-α) in the trauma group decreased from the first day to the third day and continued to increase until one week after the trauma. *Prevotellaceae_UCG_001* was correlated with TNF-a (R = 0.411, *P* = 0.033); *Lactobacillus* was negatively correlated with IL-6 (R = − 0.434, *P* = 0.024) and IL-1α (R = − 0.419, *P* = 0.030) and positively correlated with IL-8 (R = 0.391, *P* = 0.045); and *Lachnospiraceae_NK4A136_group* (R = − 0.559, *P* = 0.027) and *Muribaculaceae* (R = − 0.568, *P* = 0.024) were negatively correlated with IL-8. Severe trauma shows stress-like activities by negatively modulating intestinal microbiota and affecting certain inflammatory cytokines contributing to host health, which implies that the regulation of potentially targeted intestinal microbiota, and further mediating and maintaining the homeostasis of inflammatory cytokines, is expected to promote the accelerating recovery of the body after severe trauma.

Keywords Intestinal microbiota, Severe trauma, Inflammatory cytokines, Rat model, Bioinformatics analysis

Abbreviations

AIS	Abbreviated injury scale
ANOVA	Analysis of variance
Co., Ltd	Company of limited liability
ELISA	Enzyme-linked immunosorbent assay
g	Gram
h	Hour
IL	Interleukin

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ISS	Injury severity score
kg	Kilogram
LDA	Linear Discriminant Analysis
LSD	Least significant difference
M	Median
mg	Milligram
ml	Milliliter
MODS	Multiple organ dysfunction syndrome
NF-κB	Nuclear transcription factor-κB
No	Number
OTU	Operational taxon unit
QR	Quartile range
RPM	Revolutions per minute
SD	Standard deviation
SD rats	Sprague dawley rats
SPF	Specific pathogen-free
SPSS	Statistic package for social science
STEM	Short time series expression miner
TNF-a	Tumor necrosis factor-alpha
$\overline{X}$	Arithmetic mean
χ^2	Chi-square test

Death and disability from trauma place a heavy burden on societies and families. Trauma refers to the destruction of tissue structure, dysfunction and/or psychological damage caused by physical, chemical, biological, ecological or psychological factors[1]. Common trauma is caused by earthquakes, typhoons, fires, traffic accidents, falling from a high altitude, animal stings, blunt instrument attacks, etc.[2]. Trauma has become a serious global public health concern, ranking first among the causes of death in people aged 1–44 in developed countries. In China, there are approximately 62 million traumatic patients, and the number of traumatic deaths is up to 800,000 every year, which also ranks first among the causes of death among people under 40 years old[3]. Moreover, in the past decade, the cumulative incidence of severe trauma has been increasing globally[4]. Functional disability and labor force loss in patients with severe trauma will result in severe personal and social burdens and pose substantial challenges to healthcare systems[5,6].

In the human gastrointestinal tract, the vast majority of microbes form stable microbial ecosystems, playing crucial role in the development and life-long maintenance of host health[7]. Dynamic changes in the intestinal microbiota are involved in human growth and development, food metabolism, immune regulation and other important physiological functions[8,9]. The intestinal microbiota is inseparable from human health because the intestinal microbiota can affect the human digestive system, intestinal-brain axis, liver-gut axis, and central nervous system[10]. Posttraumatic intestinal microbiota imbalance can lead to increased intestinal permeability, leading to secondary infections (such as lung infection, wound infection, abdominal infection, implanted device infection, etc.), sepsis and even multiple organ dysfunction[11], increasing the mortality of patients[12]. The incidence of sepsis after severe trauma can reach more than 50%, and the fatality rate can be as high as 60% ~ 80% if septic shock is further developed[13]. A study suggested that sepsis development is associated with the disruption of intestinal microbiota at both the compositional and functional levels, and such enteric dysbiosis could promote organ inflammation and injury during sepsis[14]. Transfer of Gram-negative bacteria and bacterial toxins after severe trauma is the main factor leading to sepsis[15]. Multiple organ dysfunction syndrome (MODS) caused by intestinal ischemia–reperfusion is accompanied by serious bacterial imbalance, and bacterial and endotoxin translocation is also an important factor for the occurrence and development of MODS[16]. In addition, the imbalance of intestinal microbiota can affect wound repair and patients' rapid recovery through immune and inflammatory cytokines.

Studies have indicated significant intestinal microbiota differences between normal and trauma individuals. For instance, traumatic brain injury caused changes in the intestinal microbiota, and experimental stroke altered the composition of the cecal microbiota, with specific changes in *Peptococcaceae* and *Prevotellaceae* correlating with the extent of injury. These effects are mediated by noradrenaline release from the autonomic nervous system with altered cecal mucoprotein production and goblet cell numbers[17]. Study data from rodents indicate that spinal cord injury causes intestinal microbiota dysbiosis, which exacerbates intraspinal inflammation and lesion pathology, leading to impaired recovery of motor function. Postinjury delivery of probiotics can partially overcome the pathophysiological effects of gut dysbiosis, and immune function, locomotor recovery, and spinal cord integrity are partially restored by a sustained regimen of oral probiotics[18]. These results suggest a close relationship between the intestinal microbiota and inflammation of trauma.

Ample evidence indicates that the dynamic changes in intestinal microbiota in trauma patients can provide a valuable basis for the diagnosis and treatment of trauma[19] and promote prognosis[20], the effective regulation of intestinal microbiota homeostasis is a key action to promote early recovery and improve the prognosis of patients after trauma. The literature mainly focuses on major clinical problems caused by trauma, such as brain injury, spinal cord injury and inflammatory reactions[21]. However, the effect and the specific interaction mechanism of intestinal microbiota on the rehabilitation of trauma patients remains obscure, and there is still a lack of related studies on the dynamic changes in intestinal microbiota and inflammatory cytokines following a multiple severe crush and fracture trauma[22]. Therefore, studying and definitively confirming the change trajectory and relationship between intestinal microbiota and inflammatory cytokines after severe trauma is warranted.

Material and methods

This study was approved by the Animal Ethics Committee of West China Hospital of Sichuan University (No. 20220225088). This study was performed in accordance with the ARRIVE guidelines and the Declaration of Helsinki, and it was reported in accordance with ARRIVE guidelines.

All methods were performed in accordance with the relevant guidelines and regulations.

Experimental animals and groups

The experimental animals were 75 male specific pathogen-free (SPF) sprague dawley (SD) rats aged 8 weeks, weighing approximately 200 ~ 220 g, purchased from Chengdu Enswell Laboratory Animal Technology Co., Ltd. The rats were fed at a temperature of (25 ± 2)°C, relative humidity of 50% ~ 70%, air change times of 10 times/hour, with 12-h light and dark cycles. The rats had free access to food and water. The rats were randomly divided into two groups by computer: trauma group (experimental group) (n = 50) and nontrauma group (control group) (n = 25). (Fig. 1). All cage tools, bedding materials, and feed were autoclaved. During the experiment, the experimental operations, such as water change, feed addition, and pad change, were all carried out in an ultraclean workbench.

Random numbers were generated by the computer to implement allocation hiding (SPSS 26.0 was used for generating the random numbers). The serial numbers of the rats to be included in the study were then assigned by computer. The rats were successively enrolled in the study by researchers according to the serial numbers and the order of inclusion. Data analysts were blinded in this study.

Establishing the rat model of crush and fracture trauma

After 5 days of adaptive feeding, the rats were anesthetized by abdominal injection of 3% pentobarbital sodium at 40 mg/kg body weight. In the experimental group, a multiple severe crush and fracture trauma model was constructed by referring to the patient Injury Severity Score (ISS) (ISS > 16 scores) (Supplementary Table S1)[23]. The triangular area between the xiphoid process and both kidneys was accurately located with a homemade sleeve device as the impact area. A 200 g standard balance weight was used to free fall from a height of 50 cm along the homemade sleeve to hit the target area, resulting in abdominal organ impact trauma. The tibia and fibula were broken with a rongeur, resulting in unilateral lower limb fracture trauma. When the ISS component score of the trauma rat model was greater than 16, the trauma rat model was considered a severe trauma model. In our preliminary experiment, 5 rats were established with a severe trauma model, and all of them met this condition after anatomical examination, $ISS = AIS1^2 + AIS2^2 + AIS3^2 = AIS1(\text{liver lacerations} = 4\ \text{scores})^2 + AIS2\ (\text{tibiofibula fractures} = 2\ \text{scores})^2 + AIS3\ (\text{superficial body laceration} < 10\ \text{cm} = 1\ \text{score})^2 = 21$ scores. In subsequent formal experiments, animal models were not dissected and examined, and severe trauma rat models were established according to the standard practice of a preexperiment. The control group received the same anesthesia but did not establish the severe trauma model. After modeling, the rats in the two groups were returned to their cage for rest and fed conventionally. The trauma modeling was established by two trained researchers.

Sample collection

The rats' conditions were observed and recorded. The rectum of the rats was massaged at 0 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h to stimulate defecation and collect fresh feces. The fecal samples were placed into sterile cryopreservation tubes, stored in a -80 °C refrigerator, and sent for inspection in time. At the same time, 0.5 ml blood was collected from the tail vein of rats with a 24 G indwelling needle, and the serum was collected for inflammatory cytokines analysis (IL-1α, IL-6, IL-8, and TNF-α) after centrifugation at 3500 RPM for 10 min. The collected serum was temporary stored at -80 °C and inspection in time. At 168 h, the animals were anesthetized by intraperitoneal injection of 3% pentobarbital sodium at 40 mg/kg weight, and then the rats were euthanized by abdominal aorta bloodletting. The rat carcasses were handed over to the Animal Experiment Center of West China Hospital for harmless treatment.

Evaluation methods of the measurements

1. General condition and changes in the intestinal microbiota of rats after trauma: 1) General condition of rats, including observation of mental state, activity, appetite, stool and other conditions, especially posttraumatic complications and mortality. 2) Changes in intestinal microbiota were analyzed by metagenomics, amplicon analysis (16S rRNA high-throughput sequencing) and bioinformatics analysis, including operational taxon unit (OTU) clustering, species accumulation curve, and alpha and beta diversity analysis. After 16S rRNA high-throughput sequencing with STEM (Short Time—series Expression Miner) software according to the "0 h"—>"24 h"—>"48 h"—>"72 h"—>"96 h"—>"120 h"—>"144 h"—>"168 h" order trend analysis, a significant module trend chart and cluster heatmap were drawn. 3) The changes in intestinal microbiota abundance at the genus level were compared between the experimental group and the control group at 0 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h after trauma.
2. Measurement of inflammatory cytokines: The levels of the proinflammatory cytokines interleukin-1α (IL-1α), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) were measured by enzyme-linked immunosorbent assay (ELISA), which reflects the influence of trauma on the inflammatory response.

Intestinal microbial gene detection and bioinformatics analysis were carried out by OE Biotech Co., Ltd (Shanghai, China).

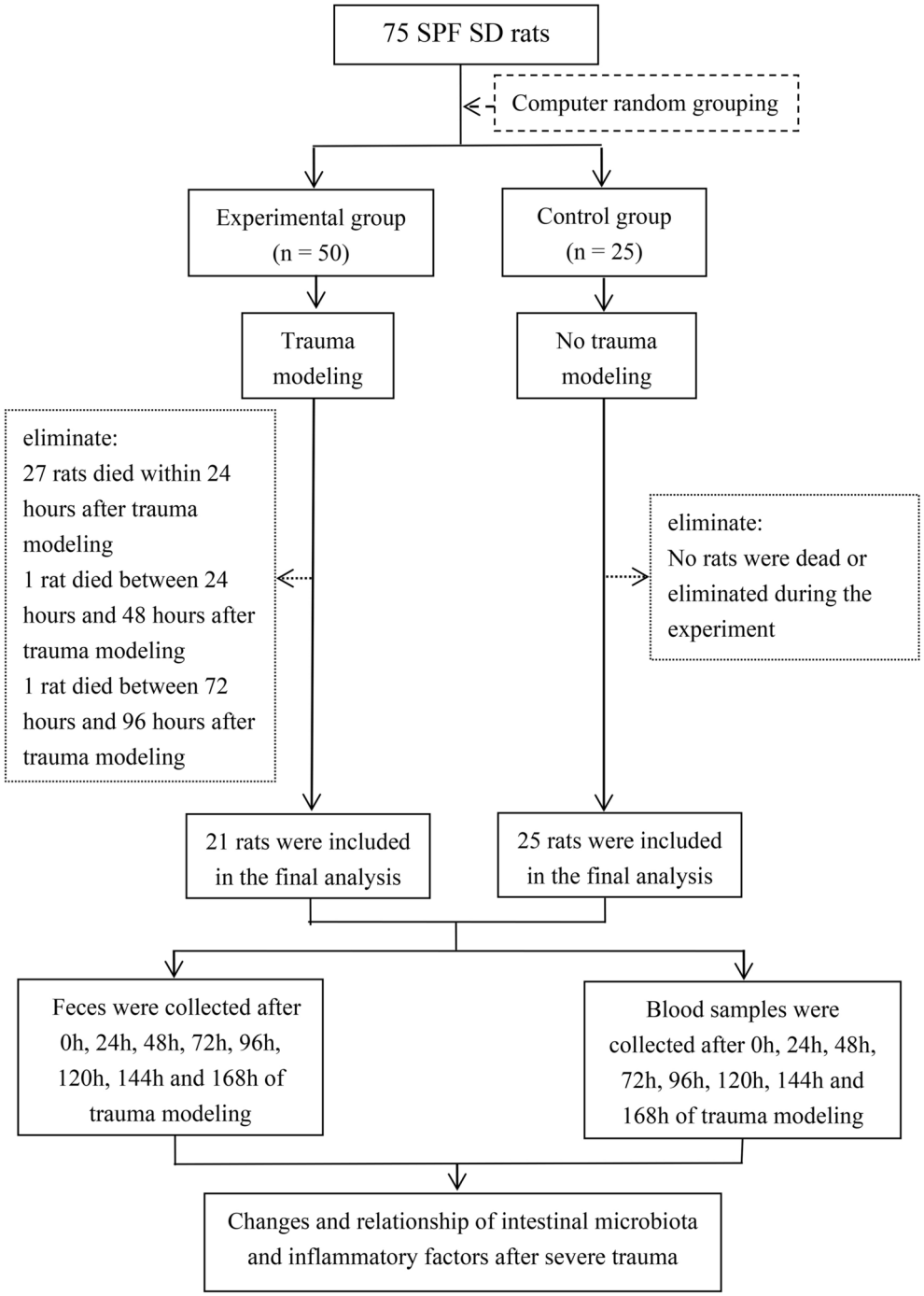

Fig. 1. Experimental flow chart.

Statistical analyses

SPSS 26.0 was used for statistical analysis, a *P*-value of <0.05 was considered statistically significant. The mean ± standard deviation ($\overline{X} \pm SD$), median (M) and Quartile Range (QR), frequency or rate was used for the statistical description. Student's t-Test, Wilcoxon test, ANOVA, LSD, Kruskal–Wallis H test, chi-square test (χ^2), rank sum test or Fisher's test was used for statistical inference. Pearson or Spearman correlation analysis was used to analyze the correlation between intestinal microbiota and inflammatory cytokines. Multiple hypothesis test LEf Se, SPSS (Alpha analysis) and PERMANOVA (Beta analysis) were used for microbiome sequencing analysis.

Results

The composition and abundance change trend of intestinal microbiota in rats after severe trauma

A total of 21 rats were included in the experimental group (trauma group), and 25 rats were included in the control group (nontrauma group) (Fig. 1). After 16S rRNA high-throughput gene sequencing analysis of the intestinal microbiota of rats, the top 15 dominant bacterial genera were identified, among which *Muribaculaceae*, *Prevotella* and *Alloprevotella* accounted for the largest proportion. *Muribaculaceae* [LDA(Linear Discriminant Analysis)-value = 4.814, $P = 0.014$] in the experimental group was slightly higher than that in the control group, while *Prevotella* (LDA-value = 5.235, $P = 0.020$) and *Alloprevotella* (LDA-value = 4.443, $P = 0.015$) were slightly lower in the experimental group than that of the control group (Figs. 2 and 3). The original 16S rRNA sequencing data for intestinal microbiota of rats have been deposited in the NCBI database under accession identification code PRJNA906715 (Release date: 2022-12-07), these SRA records would be accessible with the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA906715.

Specifically, the abundance of *Muribaculaceae*, *Lactobacillus*, *Prevotellaceae_UCG−001*, *Lachnospiraceae_NK4A136_group*, *Prevotellaceae_Ga6A1_group*, *Clostridia_UCG−014*, *[Eubacterium] _Coprostanoligenes_group*, *Butyricimonas*, *Roseburia*, and other intestinal microbiota in the experimental groups was higher than that in the control group ($P < 0.05$). The abundance of *Prevotella*, *Alloprevotella*, *Bacteroides*, *Parasutterella*, *Ruminococcus*, and *Prevotellaceae_NK3B31_group* was lower than that of the control group ($P < 0.05$) (Fig. 4).

The comparison of alpha and beta diversity between the two groups was shown in Figs. 5 and 6. It can be seen from the comparison of alpha diversity that the diversity of intestinal microbiota in the experimental group (traumatized rats, n = 21) was lower than that in the control group (non-traumatized rats, n = 25) over time (Fig. 5). Additionally, from the comparison of beta diversity, it can be seen that beta diversity differences between the two groups increased gradually with the extension of time, it was greater individual differences in the experimental group and smaller individual differences in the control group, and the intra-group differences in the experimental group were higher than those in the control group (Fig. 6).

During the one-week observation period, the diversity of intestinal microbiota in the control group was higher than that in the experimental group (Fig. 7).

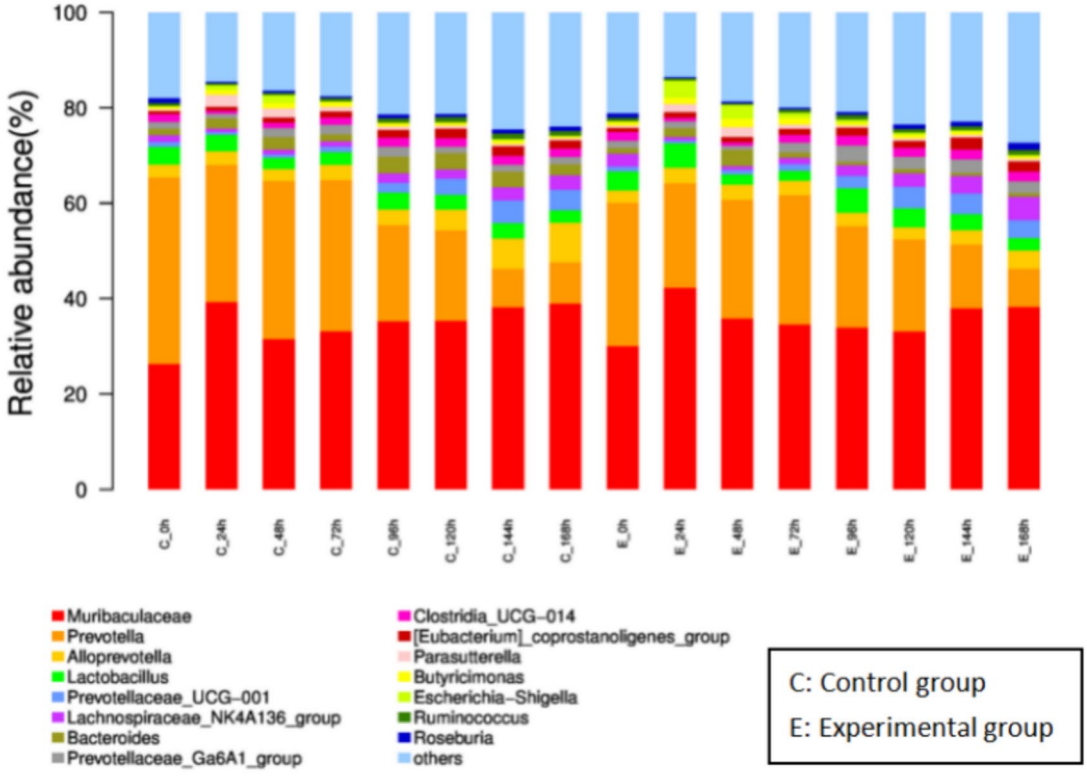

Fig. 2. Comparison of the composition and abundance change trend of intestinal microbiota at genus level between the experimental group (traumatized rats, n = 21) and the control group (non-traumatized rats, n = 25). Different colors represented different intestinal microbiota, and each color bars represented the composition ratio of intestinal microbiota relative abundance, and the different color bars represented the changing trend of intestinal microbiota abundance of traumatized and non-traumatized rats at different time points.

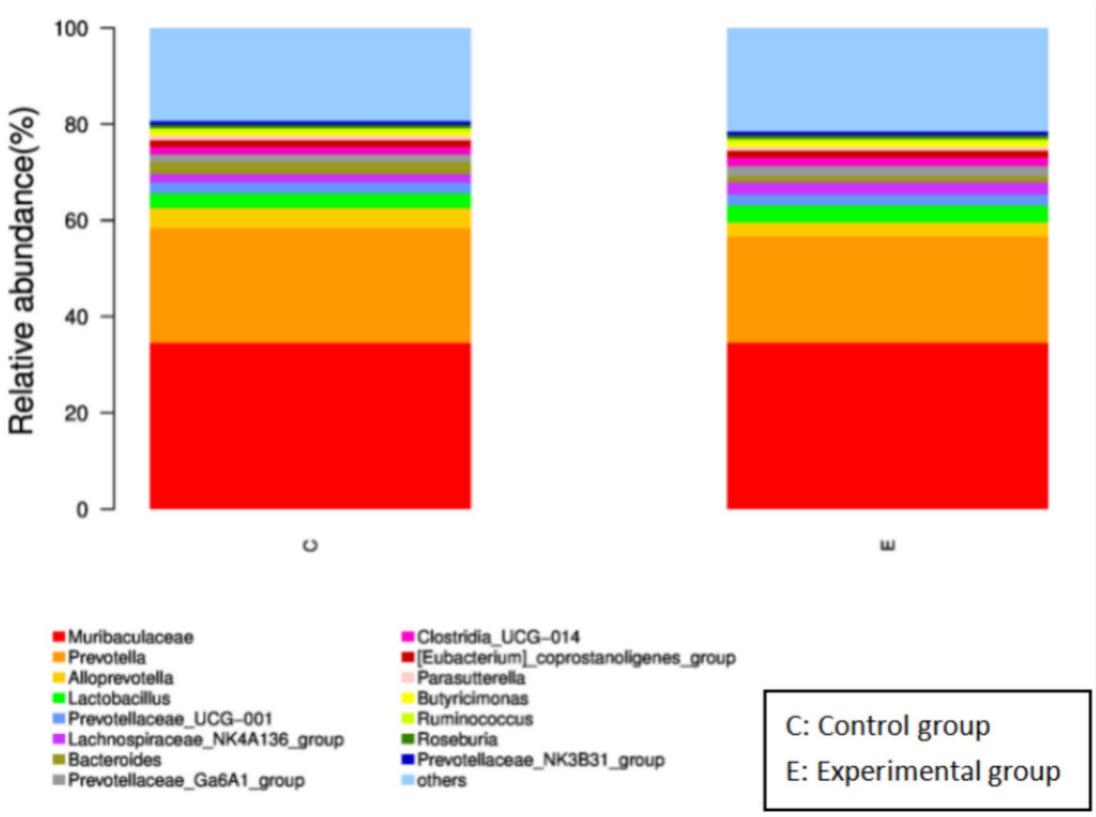

Fig. 3. Comparison of the abundance ratio of intestinal microbiota at genus level between the experimental group (traumatized rats, n = 21) and the control group (non-traumatized rats, n = 25). The different colors represented the composition ratio of the relative abundance of different intestinal microbiota in rats one week after trauma, additionally, it exhibited the comparison of the intestinal microbiota endpoint relative abundance of traumatized and non-traumatized rats.

Correlation analysis between intestinal microbiota and inflammatory cytokines

The levels of some inflammatory cytokines (IL-6 and TNF-α) in the experimental group were significantly higher than those in the control group ($P < 0.001$). IL-1α, IL-6, IL-8, and TNF-α decreased from the first day to the third day and continued to increase to one week after trauma (Fig. 8). And *Prevotellaceae_UCG-001* was negatively correlated with TNF-α (R = 0.411, $P = 0.033$), *Lactobacillus* was negatively correlated with IL-6 (R = − 0.434, $p = 0.024$) and IL-1α (R = -0.419, $P = 0.030$) and positively correlated with IL-8, and *Lachnospiraceae_NK4A136_group* (R = − 0.559, $P = 0.027$) *and Muribaculaceae* (R = − 0.568, $P = 0.024$) were negatively correlated with IL-8 (Fig. 9).

Discussion

Severe trauma induced intestinal microbiota imbalance and inflammatory cytokines' changes

A rat model of crush and fracture trauma was established in this study. And it demonstrated that severe trauma induced changes in intestinal microbiota abundance and the release of inflammatory cytokines in rats. Severe trauma induces significant physiological stress, resulting in subsequent ischemia–reperfusion injury, gastrointestinal tract interstitial cell injury[24], and increased permeability between intestinal mucosal epithelial cells[25]. The structure and function of the normal brain-intestinal axis are also affected to varying degrees[26], which induces damage and dysfunction of gastrointestinal mucosal barrier structure and intestinal microecological imbalance. The intestinal microbiota is an important part of the intestinal mucosal barrier and the largest reservoir of bacteria and endotoxin in the body; it is also an important source of posttraumatic coinfection and secondary attack of infection[12,27]. In detail, gastrointestinal mucosa is prone to produce a large number of oxygen free radicals during trauma stress reactions, which leads to gastrointestinal mucosal cell damage and ulceration and gastrointestinal motility disorder[28]. Its clinical manifestations mainly include gastroesophageal reflux, gastrointestinal paralysis, abdominal distention, and feeding intolerance caused by decreased lower esophageal sphincter tension, delayed gastric emptying[29], along with bacterial breeding and intestinal bacterial translocation, early intestinal endotoxin release, activation of the body mononuclear macrophage system, and a large release of inflammatory cytokines, causing severe systemic or organ infection, systemic inflammatory response syndrome, and even multiple organ dysfunction[13], which can be life-threatening.

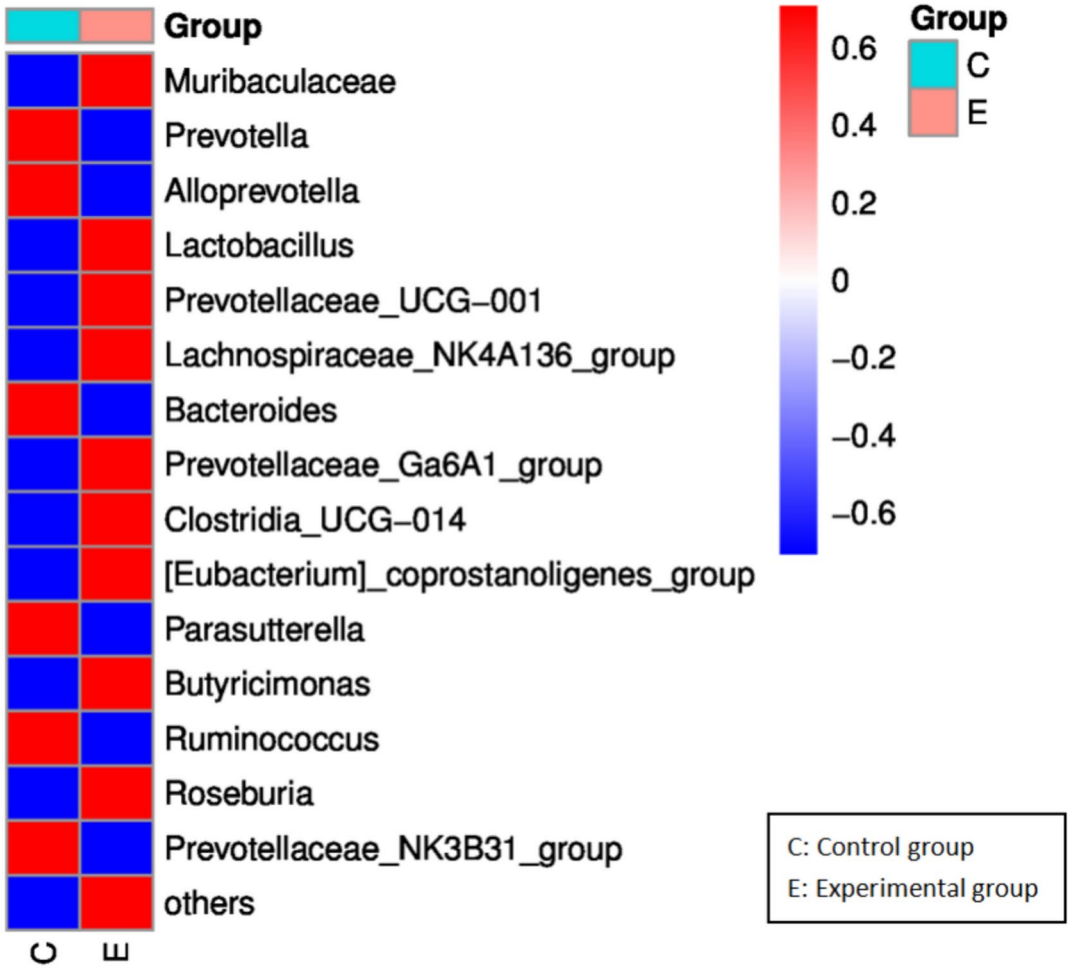

Fig. 4. Comparison of the abundance of single intestinal microbiota at genus level between the experimental group (traumatized rats, n = 21) and the control group (non-traumatized rats, n = 25). The bar on the left represented the comparison of mean intestinal microbiota abundance between traumatized and non-traumatized rats, with red representing a higher abundance and blue representing a lower abundance.

This study revealed that mechanical trauma caused pathological changes in the organism and further changed the composition and function of intestinal microbiota, resulting in a decrease in the overall abundance and diversity of intestinal microbiota (Figs. 5, 6, 7). In addition, we found the presence of specific intestinal microbiota drivers of the inflammatory response (Fig. 9). This suggests that the composition of the intestinal microbiota shortly after severe trauma exposure may play an important role in determining physiological outcomes. Previous studies have also proven that trauma causes changes in intestinal microbiota[30,31]. A similar study showed that craniocerebral injury can independently change the composition of the intestinal microbiota, and the diversity of intestinal probiotics decreases and pathogenic bacteria increase within 2 h after traumatic brain injury in mice[32]. The results of our study (Figs. 2, 3, 4) and those of similar studies indicated that the abundance of intestinal pathogenic bacteria increases[33,34] and the abundance of probiotics decreases after severe trauma[35], which leads to functional impairment and lesions of relevant organs, thus affecting the rapid recovery after trauma. A study confirmed that restoring intestinal microbiota balance and maintaining intestinal barrier function are of great significance for the treatment of diseases[36]. Therefore, clarified the change as well as the correlation between intestinal microbiota and inflammatory cytokines after trauma and explore specific targeted intestinal microbiota expected to provide a precise intervention basis for promoting the rapid recovery of patients after trauma.

The variation trend of intestinal microbiota abundance and inflammatory cytokines after trauma

During the one-week observation period, the diversity of intestinal microbiota in the experimental group was lower than that of the control group (Figs. 5, 6, 7), and the change trend of inflammatory cytokines was decreased first and then increased (Fig. 8), which indicated that trauma could cause a decrease in the overall abundance of intestinal microbiota and an increase in proinflammatory cytokines. Analyzing the reasons, it may be that trauma causes organ or tissue damage and promotes an inflammatory response, and intestinal stress injury triggers intestinal microbiota disorder and aggravates the inflammatory response[37]. In addition, it seems

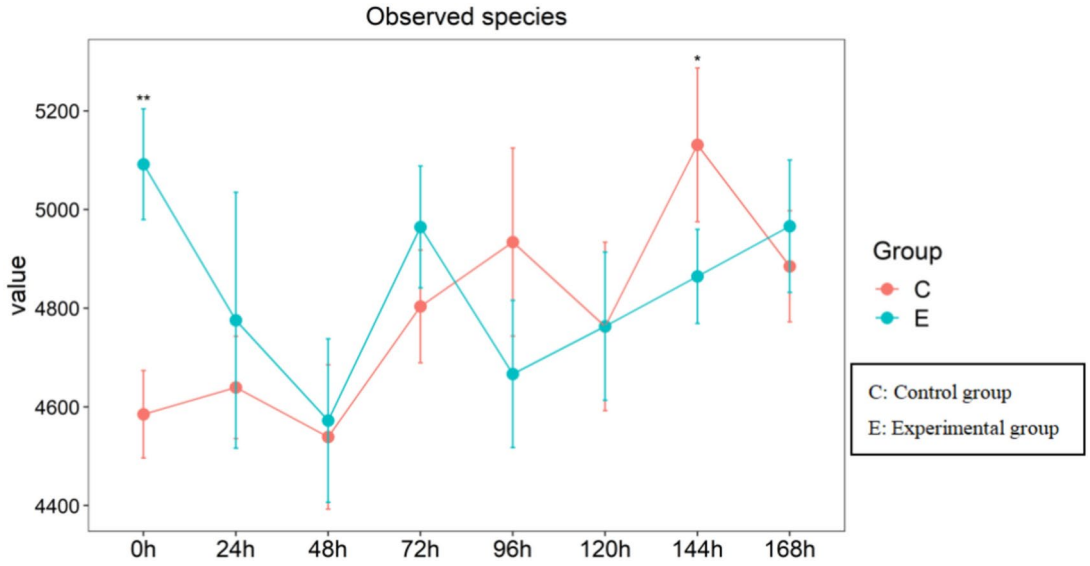

Fig. 5. Comparison of alpha diversity of the intestinal microbiota at genus level between the experimental group (traumatized rats, n = 21) and the control group (non-traumatized rats, n = 25). The values of the line graph represent: Mean ± SEM. The species richness of samples was different, and the higher the index was, the richer the species were. If * marks were marked above each time point, significant differences existed between group C and group E at this time point. Wilcoxon test was used for statistical testing. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

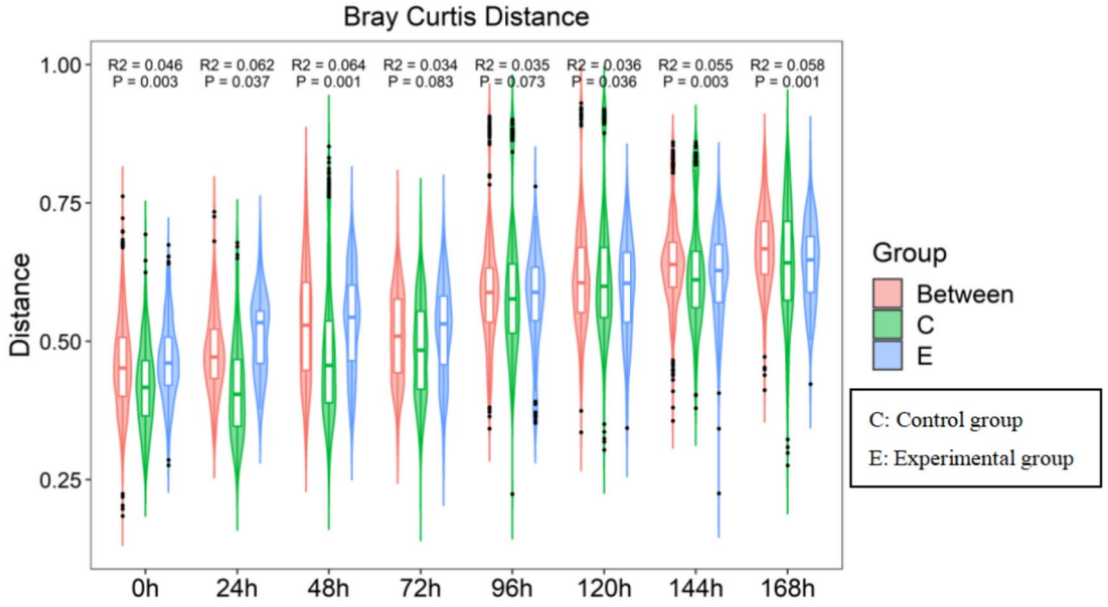

Fig. 6. Comparison of beta diversity of the intestinal microbiota at genus level between the experimental group (traumatized rats, n = 21) and the control group (non-traumatized rats, n = 25). Bray Curtis distance was used to evaluate the difference degree of species community between samples. The greater the distance value, the higher the difference degree between two samples (Y-axis). In the violin diagram, the columns "C" and "E" represent the distance between the samples within the two groups, while the column "between" represents the distance between samples between groups C and E. The horizontal line in the middle of the column represents the median value, and the higher it was, the greater the beta diversity of the group. Statistical analysis: non-parametric multivariate analysis of variance (Adonis) was used to examine the inter-group differences between group C and group E at each time point, with R^2 representing the interpretation of sample differences by different groups, the ratio of group variance to total variance. The larger R^2 was, the higher the group interpretation of difference was. *P* value, difference significance, the smaller the value, the higher the confidence.

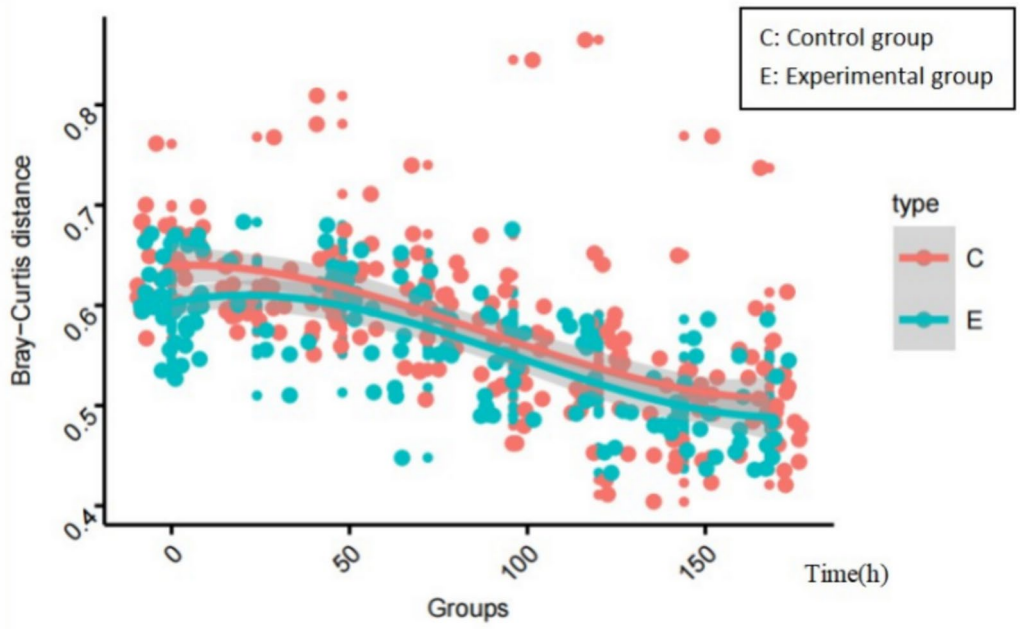

Fig. 7. The whole change trend of intestinal microbiota diversity between the experimental group (traumatized rats, n = 21) and the control group (non-traumatized rats, n = 25). Bray Curtis distance was used to evaluate the difference degree of species community between samples. The curve shows the comparative change trend of intestinal microbiota between the experimental group (traumatized rats, n = 21) and the control group (non-traumatized rats, n = 25) with the extension of trauma time. Detailedly, it took the mean value of the last time point (168 h) as the end point, and analyzed the distance between samples at each time point and the end point (the overall trend was from 0 to 168 h), and the community structure of bacteria gradually approached the result of 168 h. The height between the lines of the experimental group and the control group in this graph, which represented the difference between the sample at each time point and the 168 h time point.

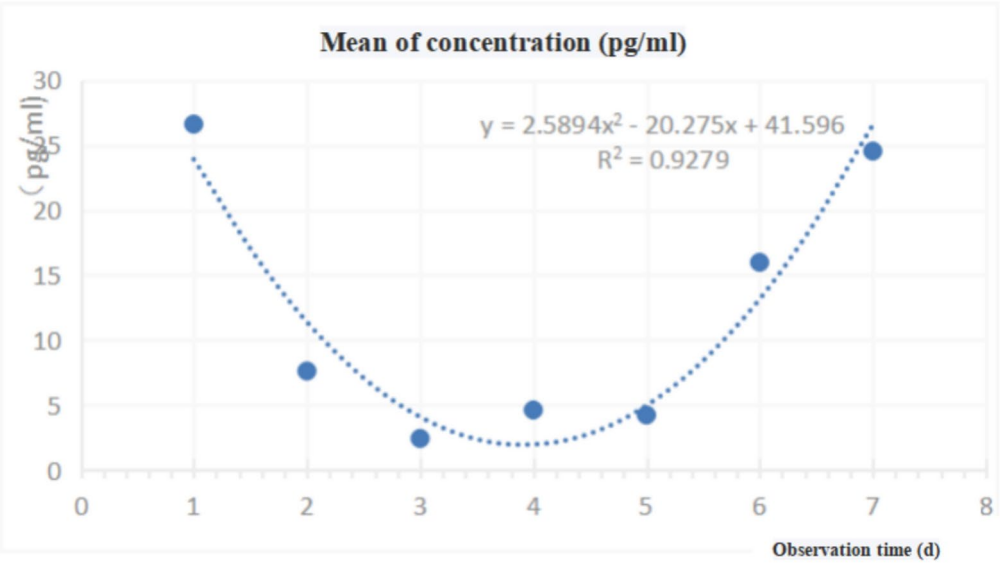

Fig. 8. Dynamic changes of inflammatory cytokines concentrations in traumatized rats (n = 21). ELISA was used to detect the level of inflammatory cytokines. The dots in the figure were the overall average concentration of inflammatory cytokines (IL-1α, IL-6, IL-8, and TNF-α) at the same time point every day within a week. The fitted curve showed the change trend of inflammatory cytokines (IL-1α, IL-6, IL-8, and TNF-α) within a week of traumatized rats.

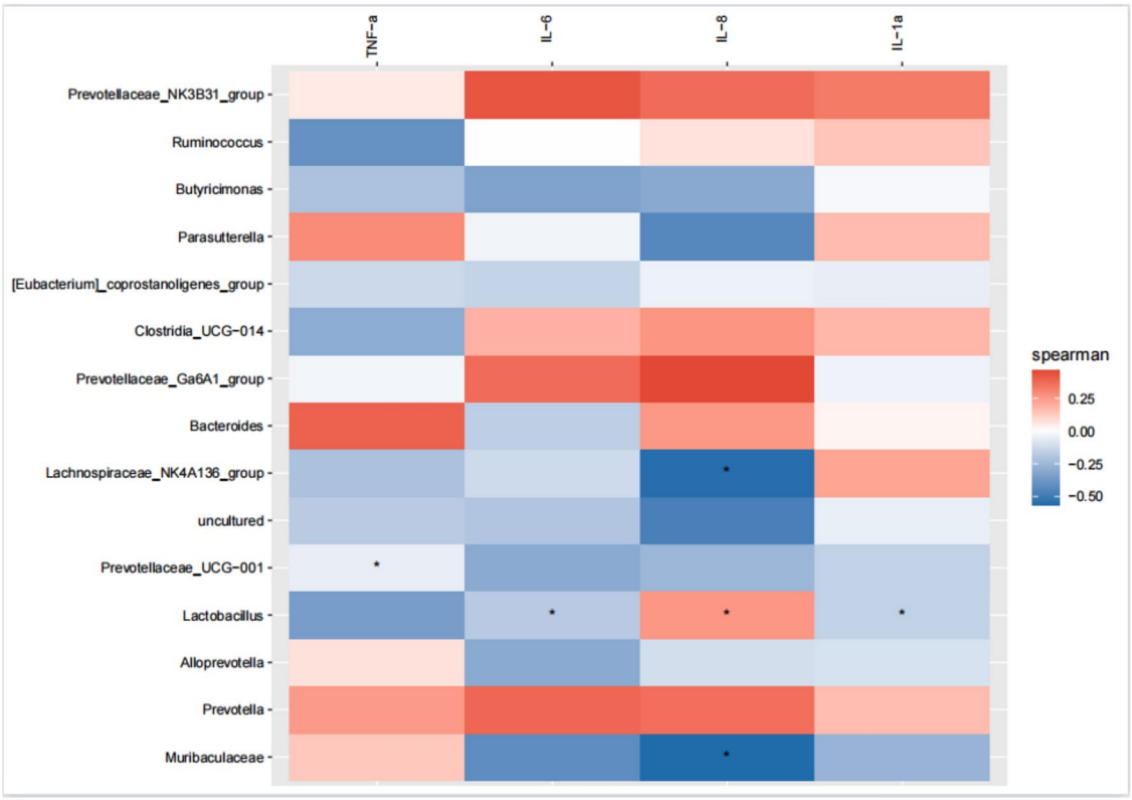

Fig. 9. Correlation between posttraumatic inflammatory factors (IL-1α, IL-6, IL-8, and TNF-α) and intestinal microbiota of traumatized rats (n = 21). Spearman correlation analysis was used to analyze the relationship between intestinal microbiota and inflammatory cytokines (IL-1α, IL-6, IL-8, and TNF-α) of traumatized rats. The grid with asterisk indicated that there was a correlation, and the correlation difference was statistically significant, *: $P < 0.05$. The more the color tends to red in the figure, the larger the absolute value of positive spearman correlation coefficient was, the closer the positive correlation was; The more the color tends to blue in the figure, the larger the absolute value of negative spearman correlation coefficient was, the closer the negative correlation was.

that the body inflammatory reaction to stimulate inflammatory cells to secrete inflammatory cytokines takes a period of time; when the inflammatory reaction continues to exist, inflammatory cytokines will be increased. Furthermore, ectopic intestinal microbiota aggravates the inflammatory response, and the overall effect is that the inflammatory cytokines first decrease and then increase[38,39].

In our study, the main types of injury in the rat model were hepatic contusion, intestinal compression and fracture. In addition to the impact of traumatic stress on gastrointestinal function and intestinal microbiota, intestinal microbiota imbalance was also caused by limited activity and inhibition of gastrointestinal function in fracture trauma. When liver function was impaired and pathological changes occurred, the abundance of intestinal microbiota in rats was decreased, and the beneficial bacteria *Bifidobacteria* and *Lactobacillus* also decreased, while the opportunistic bacteroidetes increased[40]. Cholestasis and abnormal metabolism of tryptophan were observed in rats with acute liver injury, which was characterized by increased content of conjugate bile acid-glycine deoxycholic acid and decreased content of tryptophan metabolite doquinolinic acid. These abnormalities may be related to intestinal microbiota disturbance[40]. Another study illustrated that intestinal microbiota and their metabolites can help judge the disease stage, speculate the pathogenesis and in turn carry out targeted therapy[41]. The other possibility of gut microbial dysbiosis after trauma may be because of the lifestyle change and other impacts of the trauma on a diet, as we know the critical role of diet on gastrointestinal microbial compositions. For this study, mechanical trauma was established by hitting the abdominal area, which may cause digestive organ damage, thus affecting the digestion and absorption of food and digestive tract function recovery, and further impact gut microbial homeostasis. Therefore, it is necessary to promote the recovery of posttraumatic intestinal function and restore the homeostasis of intestinal microbiota to further improve the body's rapid recovery.

Correlation between intestinal bacteria and inflammatory cytokines after trauma

As illustrated in Fig. 9, trauma can trigger an intestinal inflammatory response and reduce the diversity of intestinal microbiota. A study also revealed that severe trauma may cause systemic inflammatory response syndrome[42]. Additional evidence suggested a critical role of intestinal dysbacteriosis in intestinal trauma-related infections; for instance, an interaction effect among preoperative dysbacteriosis and early postoperative

diarrhea on total complications was observed in rectal cancer patients[43]. A similar study also demonstrated that after traumatic brain injury, the contraction of ileum smooth muscle of rats was significantly reduced, and inflammatory cytokines such as IL-1α, IL-1β and IL-17 in intestinal smooth muscle and small intestinal edema were significantly increased[44]. In addition, TNF-α could mediate damage to the tightly connected structure of cells and was the key factor in inducing the increase in permeability between epithelial cells of the intestinal mucosa[45]. The imbalance of intestinal microbiota can lead to the destruction of intestinal mucosal barrier function, resulting in intestinal infection, metabolic endotoxemia and an inflammatory response.

Emerging research indicates that gut microbiota utilize substances in the digestive tract as a source of nutrition and release various metabolites into circulation, potentially affecting the host's inflammatory balance. Certain microbiota-derived metabolites, including lipopolysaccharides, tryptophan, bile acids, and short-chain fatty acids (SCFAs), which have been proved that have the closer relationship with the host's inflammatory state[46]. Of which, the SCFAs including acetate, propionate, butyrate, isobutyrate, and isovalerate, are bacterial metabolites generated through fermentating dietary fibers. A previous study demonstrated that Luminal SCFAs as preferred energy substrates for colonic epithelia, influence epithelial barrier function, mucosal immune systems, and inflammatory responses, for example, butyrate for colitis by promoting regulatory T cells' (Tregs) formation, decreasing pro-inflammatory cytokines, activating Gpr109a to suppress inflammatory signals, and enhancing intestinal barrier. And in the severe burn injury mice model indicated that SCFAs' concentrations decreases and then changes inflammatory cytokines' expressions[9]. A study reported that dysbiosis in gut microbiota may trigger immune activation, leading to intestinal inflammation through the stimulation of inflammatory cytokines, including IL-6, IL-10, CXC chemokine receptor-3, and CXCL-11, in patients with irritable bowel syndrome. Furthermore, this study found that gut microbiota could orchestrate monocyte-like macrophage accumulation in a CCL2-dependent manner, stimulate IL-1βproduction from macrophages, and create a precancerous inflammatory environment conducive to tumorigenesis in a mouse model of colitis[47]. These studies, together with our own, elucidate the intricate causal relationship between gut microbiota, inflammatory cytokines, and the pathogenesis of inflammatory diseases and trauma. By applying a gut microbiota detect approach, we can explore whether gut microbiota casually affects inflammatory risk after severe trauma. Based on these, we tried to clarify the role of the gut microbiota in inflammatory development to eventually help to develop new treatment and nursing strategies of trauma, such as probiotic therapy, dietary modulations, and fecal microbiota transplantation.

In our study, with the prolongation of mechanical trauma, in the experimental group, the levels of some inflammatory cytokines (IL-6 and TNF-α) were higher, and the mortality rate was also higher ($P<0.001$) in rats with significant changes in inflammatory cytokines. A study proved that probiotics play a positive role in the recovery of the intestinal microbial barrier and improvement of intestinal function[48], such as *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*[49], which can benefit the intestinal and immune systems, correct intestinal microbiota imbalance, inhibit competition of potential pathogens, and improve local and systemic immunity[50]. Probiotics can also promote the secretion of anti-inflammatory cytokines, thereby inhibiting the growth of harmful bacteria in the intestine. Through the PI3K/Akt and NF-κB signaling pathways, probiotics decreased proinflammatory cytokines such as TNF-α and IL-1β and increased the antiinflammatory cytokines IL-10, thus improving the symptoms of intestinal inflammatory reactions[51]. A previous study showed that iron supplementation can increase beneficial microbiota and reduce opportunistic pathogens of the intestinal microbiota in rats[52]. Based on the close relationship between intestinal microbiota and inflammatory response. Some studies have attempted to use intestinal microbiota transplantation to treat intestinal inflammatory diseases, proving that fecal microbiota transplantation can reduce bowel permeability and thus the severity of disease by increasing the production of SCFAs, especially butyrate, which help maintain the integrity of the epithelial barrier[53]. Hence, maintaining a balanced gut microbiota helps reduce inflammation, which speeds recovery from trauma, but the mechanism involved remains to be further studied.

Limitations

There are some limitations need to be highlighted. (1) Due to the particularity of trauma and ethical considerations, rats were selected as the research object of trauma model construction in this study, and the study was not included in the population for analysis. Therefore, the applicability of the research results in the population may be biased. (2) In addition, the establishment of the trauma rat model in this study referred to the patient injury severity score (ISS) table, while the rigor and effectiveness of the scale in rats could not be judged. (3) Another shortcoming of this study was that it only studies the correlation between the changes of intestinal microbes and serum cytokines after mechanical trauma. There were currently no results to support a potential link between the gut and the circulatory system, such as bacterial metabolites in stool and blood or cytokine levels in the gut and stool, which may provide more substantial evidence to support the impact of trauma on intestinal microbial dysregulation. In the future studies should address the absence of data on bacterial metabolites and cytokine levels in different biological compartments (ex: gut and blood). (4) Several further experiments are needed for both clarification and refinement, and also to enhance the overall scientific rigor and clarity of the study, such as the inclusion of female rats, histological analysis, gut permeability, and barrier function assays, additional inflammatory and immune markers, etc. It is necessary to optimize the study design and methods in future studies and carry out relevant studies to significantly improve the impact and robustness of the work and to further improve the reliability of research evidence.

Conclusions

In conclusion, we found associations between measures of intestinal microbiota diversity and the severity of systemic and local gut inflammation in severe trauma. It is useful to formulate reasonable individualized treatment and nursing methods in focus, provide accurate nursing for regulating intestinal microecological

homeostasis after trauma, and help improve the quality of trauma care and promote the rehabilitation of patients. Future investigations with increased temporal-spatial resolution are needed to fully elucidate the effects of severe trauma on the intestinal microbiota, biological signatures of inflammation, and changes in the pathophysiology of intestinal tissue outcomes.

Data availability

All data upon which the conclusions of the paper rely are presented in the main manuscript. The original 16S rRNA sequencing data for intestinal microbiota of rats have been deposited in the NCBI database under accession identification code PRJNA906715 (Release date: 2022-12-07), this SRA records would be accessible with the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA906715.

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Author contributions

C.Q. L. designed the study, collected the data, analyzed and interpreted the data, and drafted and revised the manuscript. J. Y., H.F. R., G.N. L., Z. Y., S.L. G., Q.J. D. and X.Z. Y. contributed to animal model construction and conducted experimental work, and helped with the data collection, analysis and interpretation. H. U. and K. L. made substantive intellectual contributions to the interpretation of the data and the drafting of the manuscript. All authors read and approved the final manuscript. All authors reviewed the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

This study was approved by the Animal Ethics Committee of West China Hospital of Sichuan University (No.20220225088). This study was performed in accordance with the ARRIVE guidelines and the Declaration of Helsinki, and it was reported in accordance with ARRIVE guidelines.

Statement of methods

All methods were performed in accordance with the relevant guidelines and regulations.

Additional information

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