

Preventive effects of sodium butyrate in combination with probiotics on rats with severe burn-induced intestinal injury

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Abstract

AIM: We aimed to evaluate the preventive effects of sodium butyrate in combination with probiotics on rats with severe burn-induced intestinal injury.

MATERIALS AND METHODS: Ninety-five male Wistar rats were divided into normal control (n=15), model (n=20), sodium butyrate (n=20), probiotics (n=20) and sodium butyrate + probiotics (n=20) groups. The model of severe scald was established by using boiling water. All rats were administered with corresponding drugs once a day for consecutive 7 days. On the 7th day after modeling, all groups were anesthetized, and their intestinal tissues and blood samples were collected. Intestinal injuries were observed by HE staining. Intestinal cell apoptosis was detected with TUNEL assay. The mRNA expression levels of Bax, EFG, eNOS, iNOS, PPAR γ , IL-6, TNF- α , SICAM-1 and SVCAM-1 in intestinal tissues were measured by qRT-PCR, and the protein expression levels of Bax, EGF, NF- κ B p65, NF- κ B p52, NF- κ B p100, ERK, IKKB, eNOS, iNOS and PPAR γ were detected by Western blot.

RESULTS: Combining sodium butyrate with probiotics well repaired intestinal damage and restored intestinal barrier function. Western blot and qRT-PCR exhibited that the combination inhibited the expression of BAX but promoted that of EGF in intestinal tissues, thereby alleviating intestinal injury and activating intestinal repair. The combination significantly reduced the intestinal expression of iNOS, whereas elevated that of PPAR γ .

CONCLUSIONS: The combination of sodium butyrate with probiotics alleviated intestinal inflammation, inhibited cell apoptosis, improved intestinal repair and protected the barrier function after burn injury. Their combination elevated the expression level of PPAR γ but decreased that of NF- κ B, which mitigated intestinal inflammation, probably being the important mechanism underlying the protective effects on intestinal tissues.

KEYWORDS: burn, sodium butyrate, probiotics, intestinal inflammation

Introduction

The exudation of considerable body fluids in the early stage of severe burn injury and blood redistribution resulting from severe stress reaction significantly decrease the blood flow in intestinal tissues, lead to intestinal hypoxia as well as nutrition and energy deficiencies, thus triggering oxidative stress and inflammatory response and mediating intestinal injury. Apoptosis and necrosis of intestinal epithelial cells occur upon intestinal injury, and lead to increased permeability of intestinal mucosa, so bacteria and macromolecules

in the intestinal tract may easily enter the blood circulation through the intestinal wall, then aggravate oxidative stress and inflammatory response, and further destroy both mechanical and immunologic barriers of the intestinal tract. Finally, systemic infections take place^{1,2}. Therefore, intestinal protection plays a vital role in the treatment for serious burn injury.

Butyric acid, as a short-chain fatty acid produced through the glycolysis of dietary fibers by bacteria in the colon cavity, mainly provides energy for the colonic epithelium. Over 90% of butyric acid exists in the colon cavity in the form of anion. Sodium butyrate is a stable butyrate as the exogenous supplementation for butyrate ions³. The amount of butyric acid plummets after serious burn injury, and supplement of sodium butyrate for seriously

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burned cases can significantly inhibit intestinal inflammatory response and maintain the mechanical barrier, thus exerting apparent protective effects on intestines⁴. Probiotics also have obvious protective effects on the intestinal barrier function by regulating intestinal flora, suppressing the proliferation of harmful bacteria and correcting intestinal dysfunction⁵. Thus, combining sodium butyrate with probiotics may better protect intestines.

As reported in a previous literature⁶, abnormal up-regulation of iNOS together with abnormal synthesis and increase of NO led to oxidative stress and inflammation in intestines. Oxidative stress and inflammation result in the apoptosis of intestinal epithelial cells, damage of the intestinal barrier, bacterial invasion and translocation, and infiltration of toxins. Afterwards, they aggravate the intestinal inflammation and apoptosis of intestinal epithelial cells. It is well-established that sodium butyrate or probiotics alone can relieve intestinal inflammation and injury⁷. Accordingly, assessing the effects of single use of sodium butyrate or probiotics and their combination on intestinal injury is of great clinical significance. Meanwhile, we measured the expression levels of iNOS, eNOS, NF- κ B and PPAR γ after different treatments, aiming to explore the potential mechanism related to the protective effects of sodium butyrate and probiotics on intestines.

Materials and Methods

EXPERIMENTAL ANIMALS

Ninety-five male Wistar rats aged 8 weeks old and weighing 140-185 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (China; license number: SCXK 20060009). The rats were fed in an SPF laboratory at (20 \pm 3) $^{\circ}$ C with the humidity of (80 \pm 20)%. They were routinely fed for one week for adaptation, with a light/dark cycle of 12 h/12 h and free access to food and water. All animal experiments have been approved by the animal ethics committee of our hospital, and great efforts have been made to minimize their suffering.

MATERIALS

Probiotics were purchased from Beijing Tongrentang Chinese Medicine Co., Ltd. (China). Sodium butyrate was bought from Sigma (USA). rabbit anti-rat primary antibodies against EGF, Bax, iNOS, eNOS, PPAR γ , IL-6, TNF- α , SICAM-1, SVCAM-1, NF- κ B P65, P52, P100, IKKB, ERK and PERK were obtained from Abcam (USA). TUNEL staining kit was provided by Promega (USA). HE staining kit and BCA

protein quantification kit were purchased from Beijing Zhili Biotechnology Co., Ltd. (China).

MODEL ESTABLISHMENT

The rats were fasted one day before scald operation. All rats were weighed before scald, and intraperitoneally injected with 3% pentobarbital sodium (40 mg/kg). Then the hair on their back was cut off, and depilatory cream was applied onto this area. Three to five minutes later, the hair and depilatory cream were cleaned with cotton balls, and the back was washed with running water and dried with gauze. The rats were injected with buprenorphine (1 mg/kg) before injury operation to relieve pain. The serious scald model was thereafter established by using boiling water. The depilated area of randomly selected 80 rats was placed against a homemade scald board with an identical size of opening. Then the depilated area was completely immersed in boiling water (100 $^{\circ}$ C, 15 s) to establish a full-layer skin scald model covering 20% of the surface area. HE staining was conducted 24 h later. The normal control group (n=15) was treated with warm water (25 $^{\circ}$ C, 15s), and the other steps were the same with those of the model group. After the injury operation, the rats were kept in separate cages and given free access to food and water.

ANIMAL GROUPING AND TREATMENT

The normal control group (n=15) and burn model group (n=20) received intragastric administration of 1 ml normal saline. The sodium butyrate group (n=20), probiotics group (n=20) and sodium butyrate + probiotics group (n=20) received intragastric administration of 400 mg/kg sodium butyrate + 1 ml normal saline, 300 mg/kg sodium butyrate + 1 ml normal saline, and 400 mg/kg sodium butyrate + 300 mg/kg sodium butyrate + 1 ml normal saline, respectively. They were administered once daily for seven consecutive days. After injury, the wound surface was externally applied with iodophor daily, and maintained dry without obvious exudation.

SAMPLE COLLECTION

Blood sample collection: Blood samples were taken from the rats 7 days after injury. The rats were deprived of food and water 24 h before sample collection. After weighing, they were anesthetized by intraperitoneal injection with 40 mg/kg 3% pentobarbital sodium. Subsequently, the rats were placed on a dissecting tray. Medical scissors were used to cut the abdominal cavity open to observe the position of the heart. A disposable

blood collection needle was inserted into the heart through the septum, and the other end was inserted into blood collection tubes, 2 ml each tube. After being left still for 20 min, the samples were centrifuged at 4,000 rpm for 15 min at 4°C to take the supernatant which was divided into two portions, numbered separately, and stored in liquid nitrogen and a -20°C refrigerator prior to use.

Intestinal tissue collection: The rats were anesthetized to cut the small intestine with scissors. The sample was collected from the small intestine 2 cm below the stomach, and 4 cm of the intestinal canal was taken and divided into two parts. The intestinal tissue close to the stomach was ground by adding 1 ml/100 mg normal saline, and centrifuged at 4,000°C for 15 min at 4°C to pipette the supernatant which was numbered and placed in a -20°C refrigerator prior to use. The other part of intestinal tissue was transferred to the cryotubes, numbered and stored in liquid nitrogen.

PREPARATION OF INTESTINAL TISSUE SECTIONS AND HE STAINING

The formaldehyde-fixed small intestine was taken out and put onto a filter paper to absorb formaldehyde. A mold was added liquid paraffin and then small intestine blocks, covered and cooled at room temperature for paraffin solidification. A glass slide was placed into a liquid mixture of potassium dichromate and concentrated H₂SO₄ for over 36 h. After silica gel on the glass slide was removed, the surface was flattened. A glass sheet was cleaned with water for about 1 h, and the glass slide was soaked in ethanol for approximately 1 h, dried at 37°C and applied with polylysine to dry naturally. Then the embedded small intestine was taken, and put on a microtome with the direction adjusted to ensure that the tissue and cutting positions were consistent. Afterwards, the section thickness was adjusted to 5 µm. The slide containing intestinal tissue was placed by tweezers in warm water at 40°C, and bubbles were removed. The slide was taken out, dried in a 37°C incubator for 24 h, soaked in xylene solution 1 for 10 min, xylene solution 2 for 10 min, 95% ethanol solution for 3 min and 80% ethanol solution for 1 min respectively, rinsed by distilled water for 1 min, stained with hematoxylin for 15 min, washed gently with running water for 1-5 s to remove hematoxylin, soaked in 1% hydrochloric acid-ethanol for 1-3 s, washed with water for 30 s, returned to blue color, rinsed with running water for 10 min, washed with distilled water for about 1-3 s, stained with 0.5% eosin solution for 3 min, washed with distilled water for about 1-2 s and 80%

ethanol for 1-2 s, immersed in 95% ethanol solution for about 3 min, absolute ethanol for about 5 min, xylene solution 1 for about 3 min and xylene solution 2 for about 3 min sequentially, mounted with neutral resin, and finally observed and photographed by microscopy.

OBSERVATION OF APOPTOTIC CELLS IN INTESTINAL TISSUE BY TUNEL ASSAY

The sections were deparaffinized with xylene twice (5 min each time), washed twice with absolute ethanol (3 min each time), soaked in 95% and 75% ethanol solutions respectively (5 min each time), washed with PBS 3 times (5 min each time), digested by using 20 µg/ml proteinase K at room temperature for 25 min, washed 4 times in doubly distilled water (2 min each time), dropped PBS containing 2% hydrogen peroxide, incubated at room temperature for 5 min, and then washed twice with PBS (5 min each time). The excess residue was absorbed with absorbent paper, and the sections were added 2 drops of TdT buffer and placed at room temperature for 5 min. After the excess residue was absorbed with absorbent paper, the sections were added dropwise with 54 µl of TdT buffer, placed into a wet box and reacted at 37°C for 1 h. Subsequently, the sections were added pre-heated cleaning buffer and stop buffer at 37°C for 30 min, gently shaken at intervals, washed with PBS 3 times (5 min each time), incubated with labeled antibody in the wet box for 30 min, washed 5 times with PBS (5 min each time), added a freshly prepared 0.05% DAB solution, developed at room temperature for 6 min, and washed 5 times with water (1 min each time for the first 4 times and 5 min for the fifth time). Afterwards, the sections were counterstained with methyl green for 10 min, washed 3 times with water (put up and down for the first two times and placed still for 30 s for the last time), washed with 100% n-butyl alcohol three times, dehydrated with xylene three times (2 min each time), mounted, dried and observed under an optical microscope.

DETECTION OF BAX, EGF, ENOS, INOS, PPAR γ , IL-6, TNF-A, SICAM-1 AND SVCAM-1 MRNA EXPRESSION LEVELS BY QRT-PCR

RNAiso plus reagent and RNAiso-mate for animal tissue were purchased from Beijing Zhili Shengke Technology Co., Ltd. (China). Intestinal tissue (10 mg) was taken from each group, added 1 ml of RNAiso plus reagent and ground on ice. Then the ground tissue sample was transferred to a centrifuge tube (1.5 ml), added 1/5 volume of chloroform (about 200 µl), shaken for 10 s by

vortexing, placed still at room temperature for 5 min, and centrifuged at 12,000 \times g and 4°C for 15 min. The supernatant was transferred to a new centrifuge tube, added an equal volume of isopropanol, placed still at room temperature for 10 min, and centrifuged at 12,000 \times g and 4°C for 10 min to discard the supernatant. The precipitate was washed with 1 ml of 75% ethanol, centrifuged at 12,000 \times g and 4°C for 5 min to remove ethanol, dried at room temperature for 5 min, and dissolved by 30 μ l of DEPC water. Then 2 μ l of RNA sample was added to Nano Drop for RNA level measurement, and OD_{260/280} and OD_{260/230} were measured. After 0.8% agarose gel electrophoresis was performed, reverse transcription was carried out according to the instructions of Fermentas reverse transcription kit. qPCR was conducted to measure the expression levels of Bax, EGF, eNOS, iNOS, PPAR γ , IL-6, TNF- α , SICAM-1 and SVCAM-1 in intestines. The primer sequences are listed in Table I.

DETECTION OF BAX, EGF, NF-KB, P65, P52, P100, ERK, IKKB, ENOS, INOS AND PPAR γ PROTEIN EXPRESSION LEVELS BY WESTERN BLOT

A small intestine of about 10 mg was cut off, rinsed with PBS five times, transferred into a glass grinder, added RIPA protein lysis buffer containing protease and phosphatase inhibitor, and thereafter ground and lysed on ice for 30 min. The resulting lysate was transferred into a 1.5 mL EP tube and centrifuged at 12,000 rpm for 20 min at 4°C. After centrifugation, the supernatant was collected and stored in liquid nitrogen prior to use. The protein concentration was measured by BCA kit. Subsequently, 10 μ L of loading buffer and sample were added into a PCR tube, boiled in water for about 5 min and centrifuged at 15,000 rpm for 5 min at 4°C. After 5% SDS-PAGE was performed, the products were transferred onto a PVDF membrane. Then the membrane was blocked in TBST containing 5% skimmed milk for 4 h, incubated with primary antibodies against Bax, EGF, NF- κ B P65, P52, P100, ERK, IKKB, eNOS, iNOS, PPAR γ (1:1,000 diluted) and GAPDH (1:500 diluted) at 4°C overnight, and washed with TBST five times (5 min each time). Afterwards, the membrane was incubated with secondary antibody (1:500 diluted) at room temperature for 2 h, washed with PBST five times (5 min each time), developed by using ECL reagents, exposed to an X-ray film and analyzed with an image analysis system.

STATISTICAL ANALYSIS

All data were statistically analyzed by SPSS22.0

software and expressed as mean \pm standard deviation. Intergroup comparisons were performed by one-way analysis of variance. $P < 0.05$ was considered statistically significant.

Results

PATHOLOGICAL EXAMINATION RESULTS OF INTESTINAL TISSUE

HE staining of intestinal histopathological sections revealed that severe burns can cause intestinal inflammation, obvious congestion and edema, infiltration of a large number of inflammatory cells, necrosis of epithelial cells, and typical ulceration. Sodium butyrate and probiotics alone can alleviate the above symptoms, and reduce the degree of lesions, mainly a large number of neutrophil-based inflammatory cell infiltration, moderate and mild congestion and edema. The combined use of sodium butyrate and probiotics can significantly alleviate the lesions, including slight congestion of mucous membrane, significantly reduced inflammatory cell infiltration, well restored intestinal barrier, and a small number of proliferating fibroblasts and intestinal metaplasia. The pathological results suggest that the combination of sodium butyrate and probiotics can repair the intestinal damage after burn to some extent, and restore the intestinal barrier function (Fig. 1).

EGF MRNA AND PROTEIN EXPRESSIONS IN INTESTINAL TISSUE

qRT-PCR showed that compared with the control group, the expression of EGF mRNA in the intestinal tissue of the model group significantly decreased ($P < 0.01$), and those of sodium butyrate and probiotics groups increased ($P < 0.05$). The combined use of sodium butyrate and probiotics to increase EGF mRNA expression was significantly higher than that used alone ($P < 0.05$) (Fig. 2A). Western blot exhibited that the expression of EGF protein in the model group significantly decreased, which was significantly increased by sodium butyrate and probiotics alone or in combination (Fig. 2B). Thus, the combined use of sodium butyrate and probiotics can effectively enhance the expression of EGF in the intestine after burn.

TUNEL ASSAY RESULTS OF INTESTINAL TISSUE

Compared with the control group, the number of stained cells in the intestinal tissue of the model group significantly increased ($P < 0.05$). The use of probiotics or sodium butyrate alone significantly reduced the number of stained cells ($P < 0.05$), and the combined use of sodium butyrate and

probiotics was more effective ($P < 0.05$). Accordingly, the combined use of sodium butyrate and probiotics can effectively reduce the number of apoptosis in intestinal tissue cells (Fig. 3).

BAX MRNA AND PROTEIN EXPRESSIONS IN INTESTINAL TISSUE

qRT-PCR showed that compared with the control group, the expression of Bax mRNA in the intestinal tissue of the model group significantly increased ($P < 0.05$), and those of sodium butyrate and probiotics groups decreased ($P < 0.05$). The combined use of sodium butyrate and probiotics to inhibit Bax mRNA expression was significantly higher than that either alone ($P < 0.05$) (Fig. 4A). Western blot exhibited that the expression of Bax protein in the model group significantly increased, which was significantly decreased by sodium butyrate and probiotics alone or in combination (Fig. 4B). Hence, the combined use of sodium butyrate and probiotics can effectively inhibit the expression of Bax in the intestine after burn.

INOS AND ENOS MRNA AND PROTEIN EXPRESSIONS IN INTESTINAL TISSUE

qRT-PCR exhibited that compared with the control group, the expression of iNOS mRNA in the intestinal tissue of the model group significantly increased ($P < 0.05$), and that of eNOS mRNA was not significantly different ($P > 0.05$). Probiotics and sodium butyrate alone inhibited iNOS mRNA expression significantly ($P < 0.05$), but their combined use worked more effectively ($P < 0.05$) (Fig. 5A). Western blot showed that the expression of iNOS protein in the model group significantly increased, which was significantly decreased by probiotics and sodium butyrate alone or in combination. The protein expressions of eNOS were similar (Fig. 5B). Therefore, burns activated the expression of iNOS in the intestine and did not affect the expression of eNOS. The combined application of sodium butyrate and probiotics can effectively inhibit the expression of iNOS in the intestinal tract after burn.

NF- κ B EXPRESSION IN INTESTINAL TISSUE

Burns increased the expression of pERK and P65. Burns did not affect the expression of P100 and P52 downstream of the NF- κ B pathway, but increased the expression of IKK β , the downstream molecule of the NF- κ B classical pathway. Individual use or their combination reduce the protein expressions of pERK, P65 and IKK β , with the effect of the latter being more obvious. Burns activated the NF- κ B

pathway via the classical pathway, and combining sodium butyrate with probiotics effectively inhibited the expression of NF- κ B in the intestinal tract after burn control (Fig. 6).

EXPRESSIONS OF INFLAMMATORY FACTORS IN INTESTINAL TISSUE

qRT-PCR showed that compared with the control group, the mRNA expressions of TNF- α , IL-6, sICAM-1 and sVCAM-1 in the intestinal tissue of the model group significantly increased ($P < 0.05$), which were suppressed by probiotics and sodium butyrate alone ($P < 0.05$) or more effectively by their combination ($P < 0.05$) (Fig. 7). Thus, the combination of sodium butyrate and probiotics inhibited the mRNA expressions of intestinal inflammatory factors by regulating the NF- κ B pathway.

PPAR γ EXPRESSION IN INTESTINAL TISSUE

qRT-PCR showed that compared with the control group, the expression of PPAR γ mRNA in the intestinal tissue of the model group significantly decreased ($P < 0.05$), which was increased in the sodium butyrate or probiotics group ($P < 0.05$). The ability of sodium butyrate plus probiotics to increase PPAR γ mRNA expression was significantly higher than that used alone ($P < 0.05$) (Fig. 8A). Western blot revealed that the expression of PPAR γ protein in the model group significantly decreased, which was significantly increased by sodium butyrate and probiotics alone or in combination (Fig. 8B). Accordingly, the combined use of sodium butyrate and probiotics can effectively enhance the expression of PPAR γ in the intestine after burn, which competitively inhibited the activity of NF- κ B and indirectly suppressed the intestinal inflammatory response.

Discussion

Animal experiments have found that severe burns can cause apoptosis of intestinal epithelial cells, and impair intestinal barrier function, leading to increased permeability of the intestinal tract, changes in intestinal tissue structure, and intestinal inflammation⁸⁻¹⁰. Therefore, the observation of the pathological state of the intestine can directly reflect changes in intestinal structure and function. Intestinal permeability and structural changes are caused by apoptosis of the cells under signal stimulation after stress response. Apoptosis often triggers DNA breaks. EGF can protect the gastrointestinal mucosa, enhance intestinal epithelial cell proliferation, and repair the intestinal tract¹¹. Animal studies have confirmed that EGF can

promote the healing of intestinal ulcers. The level of serum EGF in patients with active intestinal ulcer was lower than that in healthy people, and its content was related to intestinal barrier function. In addition, it can also promote the uptake and utilization of sodium butyrate in the intestine. However, some studies have shown that EGF transcription level is low in normal cells, but its expression can be increased in injury, hypoxia and oxidative stress¹²⁻¹⁴. In necrotizing enterocolitis, it is found that the serum expression is low, and EGF in breast milk can alleviate the occurrence of this disease, suggesting that long-term onset can induce EGF production, and also cause EGF deficiency¹⁵. Moreover, the level of Bax mRNA in necrotizing enterocolitis was significantly increased, and the level of Bcl-2 transcription was significantly reduced. BAX is a member of the BCL-2 family, which plays a role in promoting apoptosis in the BCL-2 gene family. Over-expression of BAX can promote apoptosis, while BCL-2 has a protective effect on cells. A decrease in BCL-2 or an increase in BAX often means apoptosis in cells. Our study found that severe burns can inhibit the expression of EGF in intestinal tissues, which is different from other studies¹²⁻¹⁴. It may be that after seven days of intestinal stress, intestinal necrosis is accelerated, leading to a serious shortage of cells capable of expressing EGF in intestinal epithelium during stress induction. Pathological sections showed intestinal inflammation, marked congestion and edema, typical ulceration, infiltration of a large number of inflammatory cells, and necrosis of epithelial cells in severe burns might be associated with insufficient active cells at the necrotic site. Additionally, a large number of apoptotic cells were found in the lesions of the small intestine after severe burn through TUNEL staining. Further detection revealed that burns enhanced the expression of the pro-apoptotic protein bax. Sodium butyrate or probiotics alone can effectively enhance the expression of EGF and reduce the permeability and tissue structural destruction of the intestinal tract caused by burns TUNEL staining also revealed that they could effectively inhibit the occurrence of apoptosis. Meanwhile, the expression of the apoptotic protein BAX was down-regulated. Their combined use can effectively up-regulate the expression of EGF, down-regulate the expression of BAX, and improve intestinal damage.

In this study, burns could activate iNOS expression and transcription in the intestine, but did not affect the expression and transcription of eNOS; Sodium butyrate and probiotics alone could inhibit iNOS expression and transcription, and the

combined use could inhibit the expression of iNOS more effectively. The abnormal expression of iNOS in the intestine was the main factor of NO increase, and the combination of sodium butyrate and probiotics reduced the abnormal production of NO by inhibiting the abnormal expression of iNOS, thereby inhibiting intestinal inflammation and reducing intestinal damage. Intestinal barrier destruction is caused by inflammation, and NF- κ B is an important inflammatory signaling pathway¹⁶. Therefore, intestinal inflammation caused by burns is not only manifested in apparent edema and intestinal mucosal damage. We also observed the changes of inflammatory pathway and its downstream inflammatory factors. Inflammatory molecules IL-6, TNF- α , SICAM-1, and SVCAM-1 in the intestine were significantly increased in post-burn transcript levels, and that sodium butyrate or probiotics alone could effectively inhibit the production of inflammatory factors, and the effect of the combined use was better than that of single use. Besides, we found that burn stimulation activated the MAPK-NF- κ B pathway, ERK and PERK were significantly up-regulated, and NF- κ B P65 was subsequently up-regulated, and the downstream molecule IKK β of its classical pathway was up-regulated, but the downstream molecules of PMP and P52 of the bypass pathway were not significantly changed. It is suggested that burns activated NF- κ B through the classical pathway and trigger an inflammatory response. Sodium butyrate alone or in combination with probiotics regulates intestinal inflammation and protects the intestines through classical pathways. PPAR γ can bind to p65/p50 to form a transcriptional inhibition complex, which reduces the transcriptional activity of DNA, and can also competitively bind to P300 and CBP to inhibit the activity of NF- κ B. Therefore, PPAR γ can also reduce the expression of inflammatory factors by inhibiting NF- κ B. Herein, burns can reduce the expression of PPAR γ in the intestine. By using sodium butyrate and probiotics alone or in combination, it can effectively increase the expression of PPAR γ and indirectly reduce intestinal inflammation caused by burns.

Conclusions

In summary, the burns can cause up-regulation of iNOS expression, inhibit the expression of PPAR γ , enhance the expression of NF- κ B in the inflammatory pathway and activate the transcription of inflammatory molecules, and the combined use of sodium butyrate and probiotics can effectively inhibit the expression of iNOS, enhance the expression of PPAR γ , then inhibit the

expression of NF- κ B, and reduce the intestinal inflammatory response. This may be an important mechanism for the effective protection of the intestinal tract by the combination of sodium butyrate and probiotics.

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TABLE I. Primer sequences.

Gene		Primer sequence
iNOS	Upstream primer	5'-GCCTCCCTCTGGAAAGA-3'
	Downstream primer	5'-TCCATGCAGACAACCTT-3'
eNOS	Upstream primer	5'-TGACCCTCACCGATACAACA-3'
	Downstream primer	5'-CTGGCCTTCTGCTCATTTTC-3'
Bax	Upstream primer	5'-ACCAAGAAGCTGAGCGAGTG-3'
	Downstream primer	5'-CCCAGTTGAAGTTGCCATCA-3'
GAPDH	Upstream primer	5'-CTCTGCTCCTCCCTGTTCT-3'
	Downstream primer	5'-CTTGACTGTGCCGTTGAACT-3'
IL-6	Upstream primer	5'-TGTATGAACAGCGATGATG-3'
	Downstream primer	5'-AGAAGACCAGAGCAGATT-3'
TNF- α	Upstream primer	5'-AAATGGGCTCCCTCTCATCAGTTC-3'
	Downstream primer	5'-TCTGCTTGGTGGTTTGCTACGA-3'
ICAM-1	Upstream primer	5'-TCACCTGGACAAGAAGGACTG-3'
	Downstream primer	5'-GTCAGATTAGGGGCTGGATTC-3'
VCAM-1	Upstream primer	5'-TGCTCCTGACTTGCAGCAGCACCAC-3'
	Downstream primer	5'-TGTCATCGTCACAGCAGCACC-3'
PPAR- γ	Upstream primer	5'-CCCACCAACTTCGGAATCAG-3'
	Downstream primer	5'-GGA ATGGGAGTGGTCATCCA-3'
EGF	Upstream primer	5'-GTGACCGTTTGGGAGTTGATGA-3'
	Downstream primer	5'-GGCTGAGGGAGGCGTTCTC-3'

Figure Legends

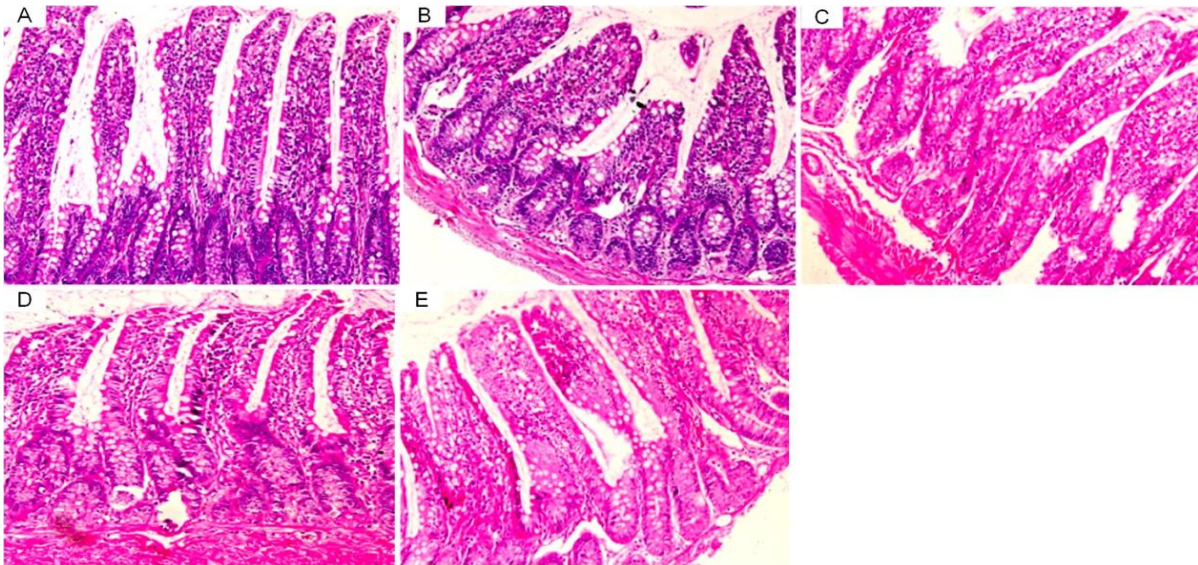


Fig. 1. Intestinal tissues of different groups 7 d after injury under a microscope (HE staining, magnification: 200 \times). A: Control group; B: model group; C: probiotics group; D: sodium butyrate group; E: probiotics + sodium butyrate group.

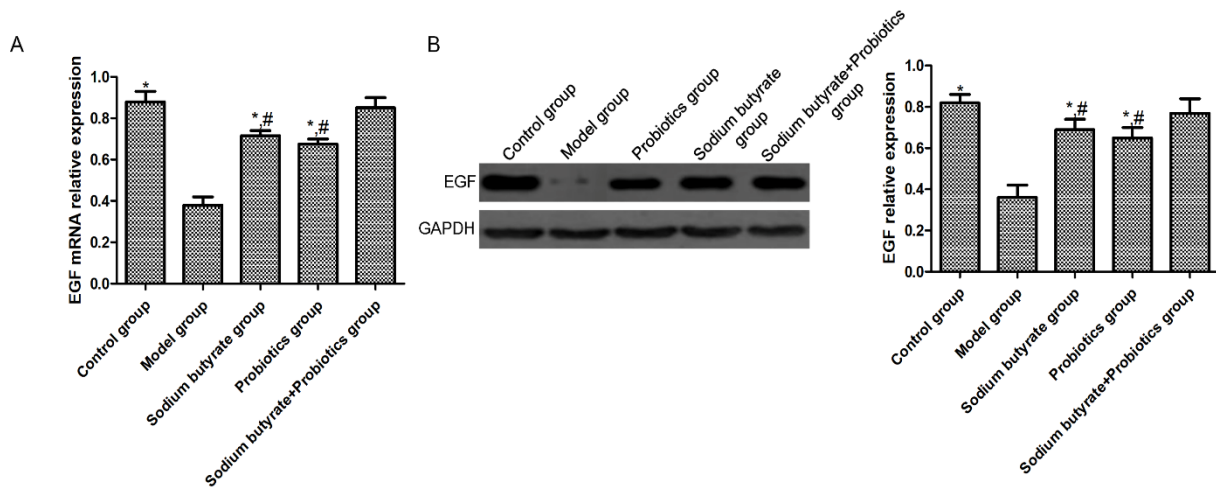


Fig. 2. EGF mRNA and protein expressions in intestinal tissue. A: EGF mRNA expression; B: EGF protein expression. *Compared with model group, $P < 0.05$; #compared with sodium butyrate + probiotics group, $P < 0.05$.

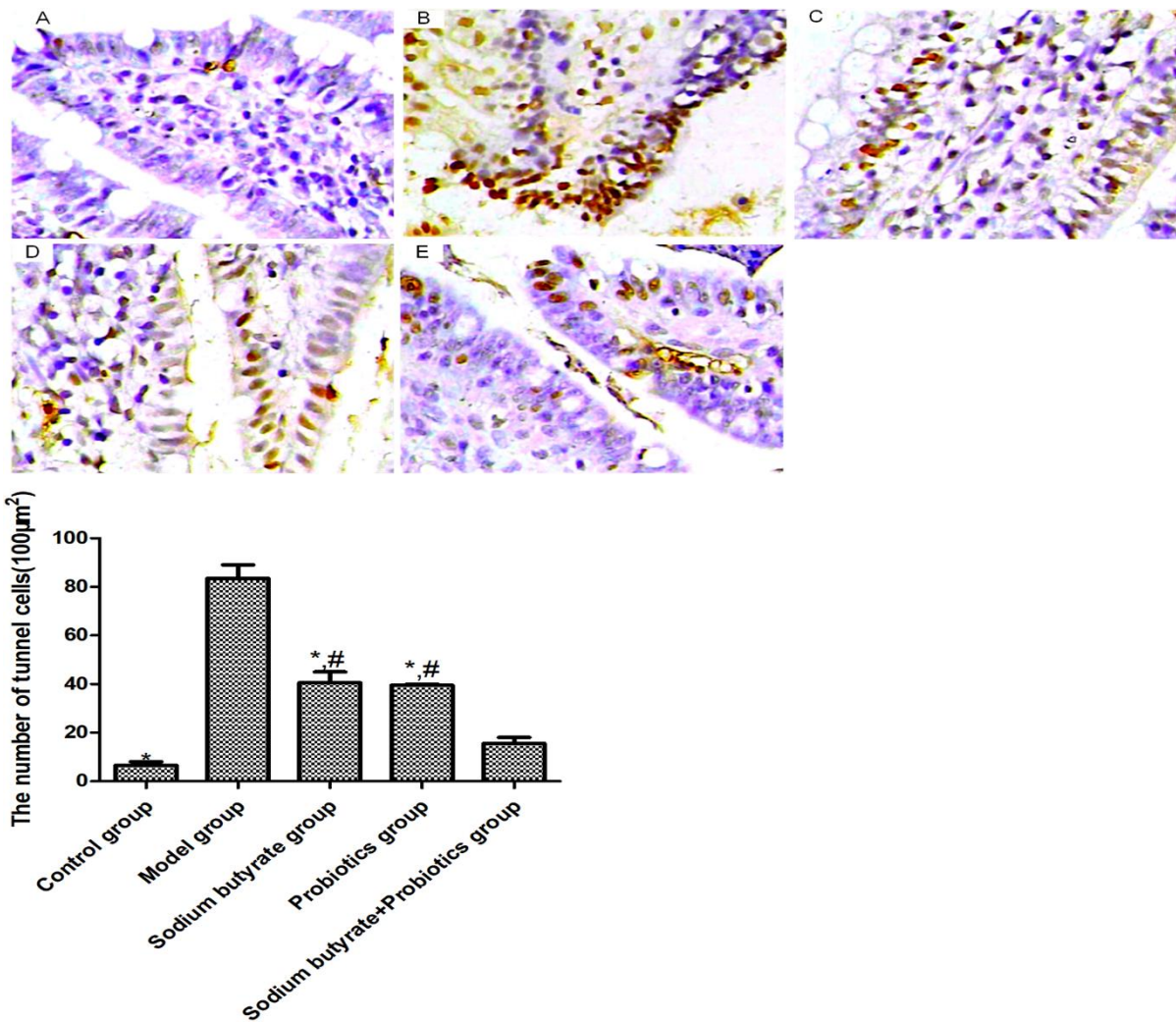


Fig. 3. Cell apoptosis in intestinal tissue detected by TUNEL assay. A: Control group; B: model group; C: probiotics group; D: sodium butyrate group; E: probiotics + sodium butyrate group. *Compared with model group, $P < 0.05$; #compared with sodium butyrate + probiotics group, $P < 0.05$.

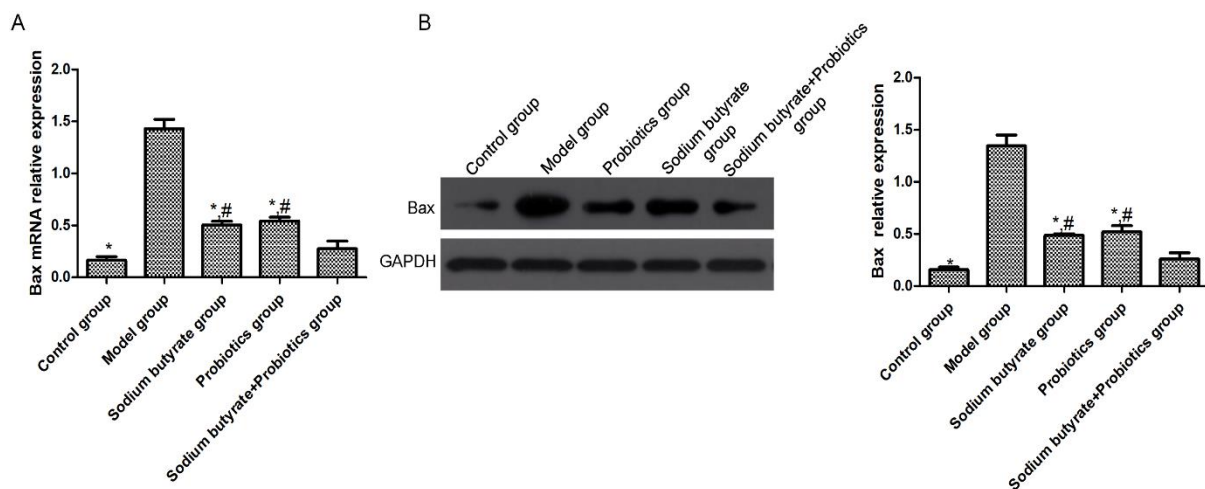


Fig. 4. Bax mRNA and protein expressions in intestinal tissue. A: Bax mRNA expression; B: Bax protein expression. *Compared with model group, $P < 0.05$; #compared with sodium butyrate + probiotics group, $P < 0.05$.

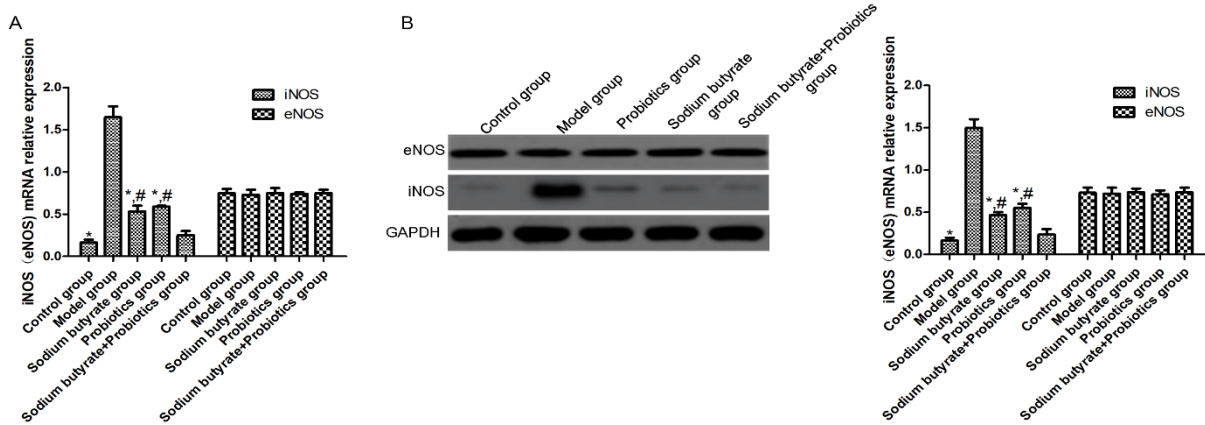


Fig. 5. iNOS and eNOS mRNA and protein expressions in intestinal tissue. A: iNOS and eNOS mRNA expressions; B: iNOS and eNOS protein expressions. *Compared with model group, $P < 0.05$; #compared with sodium butyrate + probiotics group, $P < 0.05$.

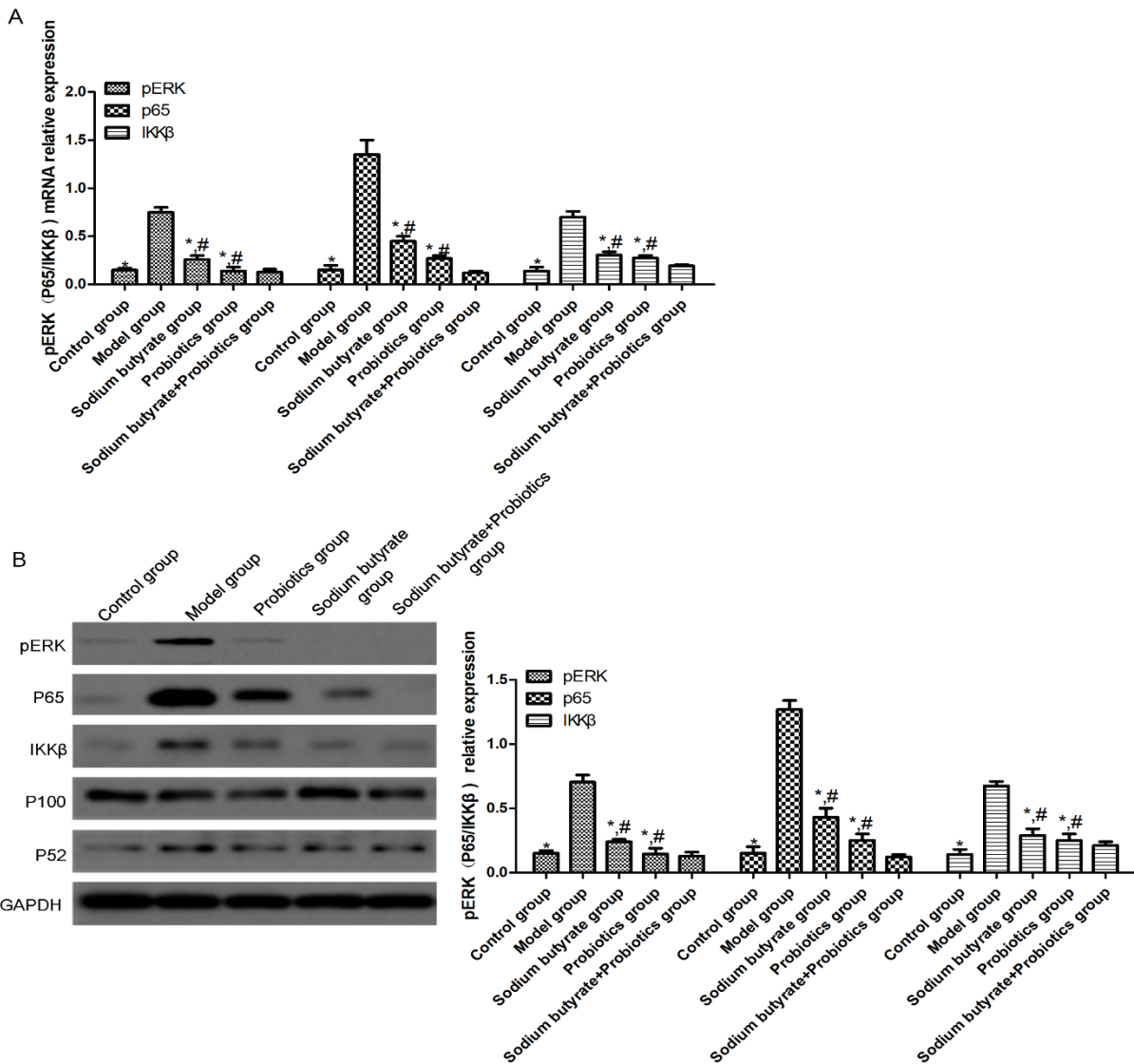


Fig. 6. NF- κ B expression in intestinal tissue. A: NF- κ B mRNA expression; B: NF- κ B protein expression. *Compared with model group, $P < 0.05$; #compared with sodium butyrate + probiotics group, $P < 0.05$.

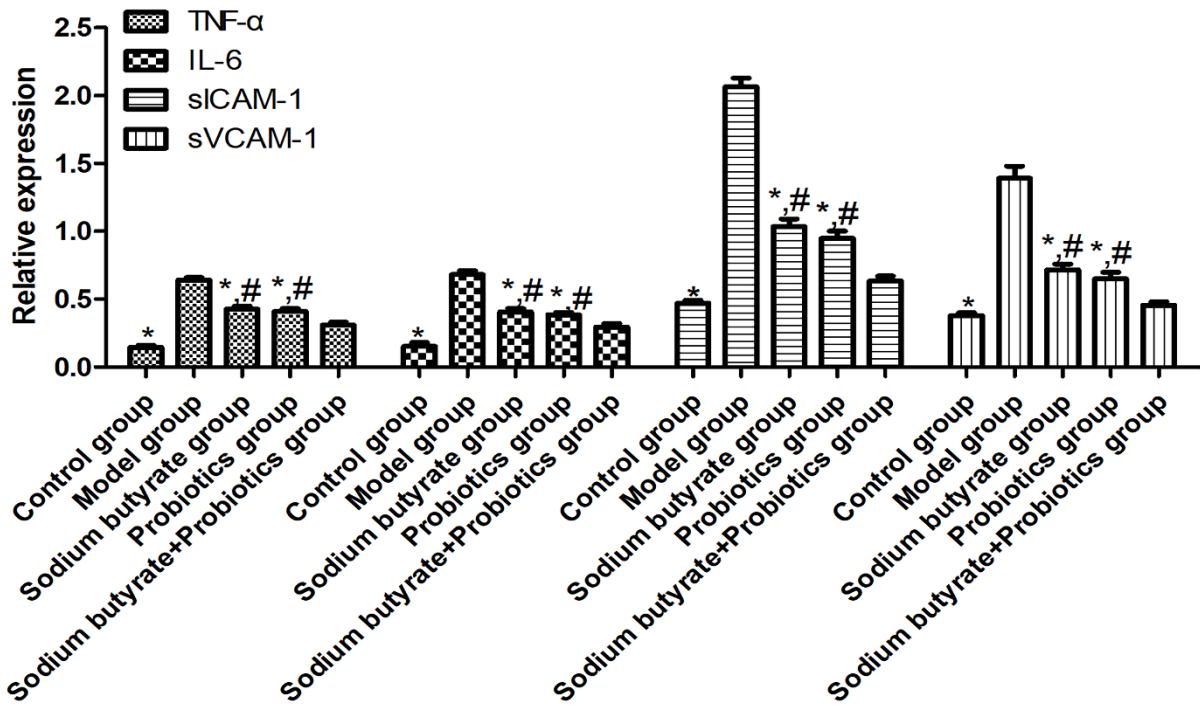


Fig. 7. Expressions of inflammatory factors in intestinal tissue. *Compared with model group, $P < 0.05$; #compared with sodium butyrate + probiotics group, $P < 0.05$.

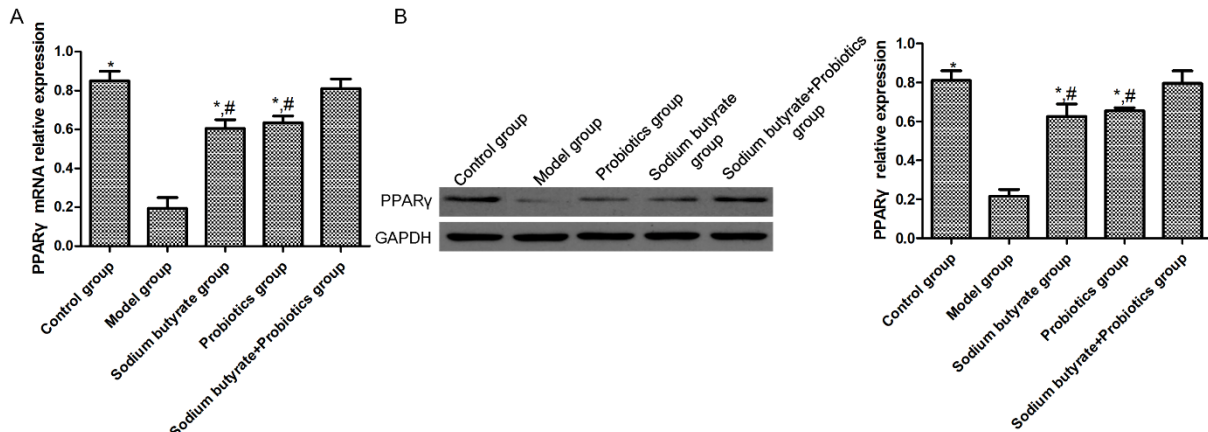


Fig. 8. PPAR γ expression in intestinal tissue. A: PPAR γ mRNA expression; B: PPAR γ protein expression. *Compared with model group, $P < 0.05$; #compared with sodium butyrate + probiotics group, $P < 0.05$.