Comparison of Effects of Aminosalicylic Acid, Glucocorticoids and Immunosuppressive Agents On The Expression of Multidrug-Resistant Genes in Ucerative Colitis

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Research Article

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Abstract

Objective: To compare the effects of aminosalicylic acid, glucocorticoids and immunosuppressive agents on the expression levels of multidrug-resistant genes in ucerative colitis (UC) patients, aiming to provide theoretical and therapeutic basis for the diagnosis, treatment and prevention of UC.

Methods: Fresh specimen of colon mucosal tissues pathological mucosal tissues under endoscopy or postoperative pathological biopsy tissues were collected from 148 UC patients and then prepared for subsequent experiment.Immunohistochemical staining was conducted to detect the distribution site and pattern of P-glycoprotein （P-gp）. The effects of ASA glucocorticoids and immunosupresive agents on P-gp were statistically compared.The expression levels of Multidrug resistance gene （MDR1）mRNA before and after corresponding treatment were quantitatively measured by using RT-PCR. In addition, the effects of three agents upon MDR1 mRNA were also comparatively analyzed.

Results: Administration of 5-aminosalicylic acid (5-ASA) drugs were not correlated with the expression of multidrug resistance genes in UC, whereas delivery of glucocorticoids and immunosuppressant drugs was positively correlated with the expression profile. The expression levels of MDR1 gene and its product P-gp were significantly up-regulated in patients who were nonresponsive to glucocorticoids and immunosuppressant agents.

Conclusions: Overall, the findings in the present study demonstrate that 5-ASA exerts no effect upon the expression levels of MDR1 and its product P-gp in patients diagnosed with UC. Nevertheless, administration of glucocorticoids and immunosuppressant drugs can up-regulate the expression levels of MDR1.

Introduction

At present, no specific treatment has been employed for ucerative colitis (UC). Use of medication is mainly adopted to alleviate the severity of relevant symptoms. Commonly-used drugs include aminosalicylic acid, corticosteroids and immunosuppressants. Amino acid drugs including willow nitrogen sulfanilamide pyridine, salad and willow nitrogen sulfanilamide pyridine (sulfasalazine, SASP), are chosen to treat UC. After oral administration, salazopyridine can be cleaved into 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP) under the action of azo reductase in the normal intestinal flora. 5-ASA is the main effective component of drug therapy [1−2]. Salazosulfapyridine is commonly utilized to treat mild and moderate UC, primarily combined with other types of drugs in severe cases. New salicylic acid drugs are widely applied in clinical practice. Azo salicylic acid, a 2 by 2 molecule of 5-ASA with azo linkage, can reach the terminal ileum, and colon bacteria in the stomach and small intestines after leaving the oral cavity, and break down in 2 min. In addition, 5-ASA plays a role in the topical anti-inflammatory mechanisms, which yields equivalent efficacy and is more tolerable compared with the willow nitrogen sulfanilamide pyridine. SASP acts as an alternative with fewer side effects and better tolerance. The following sustained-release dosage forms are available for oral administration including

Pentasa, Asacol, Salofak and Rowasa. However, the efficacy of these drugs is not significantly superior to that of SASP [3]. 4-aminosalicylic acid (4-ASA), also known as PAS, also yields equivalent clinical efficacy to prednisolone.

Recently, azathioprine has been widely employed to treat inflammatory bowel diseases, especially in refractory UC patients with hormone resistance or dependence and those complicated with fistulas. Azathioprine exerts immunosuppressive and immunomodulatory dual effects, which can prevent the proliferation and activation of T cell subsets, block the cellular response mechanism, inhibit the chemotaxis of neutrophils, and assist in decreasing the dose of glucocorticoid and extending the remission period [4]. It exerts an effect enduring for 3 to 4 months, and it can be combined with use of glucocorticoids. The dose reduction of glucocorticoids takes approximately 6 weeks. The white blood cell count should be monitored every 2-3 months on a regular basis. The incidence of myelosuppression depends upon the activity of thiopurine S-methyltransferase (TPMT), which can be used to predict the risk of myelosuppression after AZA use [5].

Inflammation is an adaptive response of the colonic mucosal immune system against invasive enteric antigens,. The production of inflammatory cytokines is necessary for eliminating pathogens. Under normal circumstances, inflammatory response will disappear immediately after the removal of pathogens. If the inflammatory response cannot be allviated, it will aggravate into refractory chronic inflammatory bowel diseases [6–11]. Biotherapy aims to block the inflammatory signaling pathway of mucosa and the inflammatory process. The biotherapeutic drugs include natural biological drugs, recombinant peptides or proteins (cytokines), antibodies, nucleic acids (antisense oligonucleotides) and gene therapy drugs, *etc*. In recent years, tumor necrosis factor (TNF) inhibitors, such as Infliximab, have been proven to yield positive outcomes.

Glucocorticoids can resist inflammation and suppress autoimmunity, which are one of the main drugs to treat UC. Clinical indications mainly consist of patients are nonresponsive to ASA drugs, and those with acute onset and severe or fulminant UC, *etc*. New glucocorticoids exert potent anti-inflammatory effects and yield few systemic adverse events [12]. Budesonide can be rapidly inactivated in the liver after being absorbed by the intestinal tract, which exerts no effect upon the serum cortisol level. Becloresone dipropionate exerts no impact upon the hypothalamus-pituitary-adrenal axis secretion, which can be administered in an enema form. In addition, it can be completely inactivated in the liver after the absorption of thiohydrogen cortisone enema, which will not lead to the imbalance of glucose, electrolyte and other metabolisms. Long-term use of such drugs is prone to side effects and cannot prevent recurrence. Hence, the drug dosage should be reduced until full withdrawal after relevant symptoms are mitigated [12].

In the present multi-center study, 148 patients with UC were selected from outpatients and inpatients of three hospitals and treated with different types of drugs. Moreover, the effects of aminosalicylic acid, glucocorticoids and immunosuppressive agents on the expression levels of multidrug-resistant genes in

UC patients, aiming to provide theoretical and therapeutic evidence for the diagnosis, treatment and prevention of UC.

Materials And Methods

Study subjects

In total, samples were obtained from 148 UC patients (study group), 83 male and 65 female, aged 16-69 years old (mean age: 42.40±13.21 years old), admitted to Outpatients and Inpatients of the First, Second and Third Affiliated Hospitals of Henan University of Science and Technology from March, 2015 to December, 2019. Among them, 58 cases were treated with ASA drugs, 53 with glucocorticoids and 37 with immunosuppressive drugs. Forty-five patients receiving electronic colonoscopy indicative of no intestinal symptoms, with a history of irritable bowel syndrome (IBS) or UC, no history of drug allergy or use of amino acid/immunosuppressant drugs or use of glucocorticoid hormone drugs were enrolled in the control group, 25 male and 20 female, aged 18-65 years old, 36.70±11.41 years old on average. All patients were followed up from the admission or discharge date to December, 2019. Colonoscopy biopsy and postoperative pathological examination are regarded as the golden standard. The comparison of general data as shown in Table 1.

Classification and staging of UC

Patients with incomplete information were excluded from subsequent experiment. In accordance with the Chinese Consensus on Diagnosis and Treatment of Inflammatory Bowel Disease constituted by Chinese Medical Association Digestive Branch in 2007 (hereinafter referred to as "07 consensus") [13], all enrolled patients were divided into mild and severe,illustrated in Table 2.

According to the course of UC, UC can be divided into initial type, chronic recurrence type, chronic persistence type and acute outbreak type. Additionally, UC can be divided into the active and remission phases according to the disease stage. The diagnosis was made by referring to the Sutherland Disease Activity Index (DAI). If the index score is lower than 2, it is classified in a remission phase; if it is higher than 2, it is classfied in an active phase, as shown in Table 3.

Lesion site

Lesion sites can be further classified as the rectum, left semicolon, broad colon and whole colon. The left semicolon refers to the flexion of the colon spleen to the rectum. The extensive colon includes the splenic flexure on the proximal colon. Lesions limited to the splenic flexure of the distal colon are classified as left semicolon lesions, and those beyond the splenic flexure but not reaching the total colonic lesion range are the extensive colon.

Diagnostic criteria

The diagnosis of UC can be confirmed by the clinical manifestations, such as diarrhea, mucous pus and hematochezia accompanied by abdominal pain, tenesmus and varying degrees of systemic symptoms;

persistent and repeated attacks; the course of UC>1 month, colonoscopic observations of continuous and diffuse distribution in distal colon, such as the rectum and sigmoid colon, mucosal vascular congestion, bleeding, edema, blurred texture, disorder or disappearance, purulent secretion adhesion, barium enema examination including intestinal edges were serrated or burr-like, multiple small filling defects on intestinal wall, rough with/or granular changes mucous membrane, shortened bowel and the pouch disappears into a lead tube, and pathological examination. Those with bacterial dysentery, amoebic dysentery, chronic schistosomiasis, intestinal tuberculosis and other infectious colitis, Crohn's disease (CD) of the colon, ischemic colitis and radioactive colitis were excluded.

Inclusion criteria

Those had not received ASA drugs, oral or intravenous glucocorticoids, glucocorticoids plus immunosuppressive agents and Chinese medicine at 4 weeks before the start of the study; Those receiving oral administration of ASA drugs after treatment: oral or intravenous administration of glucocorticoids; oral or intravenous administration of glucocorticoids plus immunosuppressive drugs, local treatment drugs such as ASA drugs and glucocorticoids, *etc*.

Prognosis and curative effect

Complete remission (disappearance of symptoms and normal mucosa by colonoscopy); effective treatment (basic disappearance of symptoms, mild mucosal inflammation or pseudopolyp formation by colonoscopy); ineffective (no improvement in symptoms, colonoscopy or pathology after treatment). All patients were divided into the effective (complete remission and effective), ineffective, and control groups.

Specimen preparation

A fresh specimen of colon mucosal tissue or pathological mucosal tissue under endoscopy or postoperative pathological biopsy tissue was immediately stored in a -80°C refrigerator for RNA extraction. After the remaining specimens were fully fixed by 10% formalin, routinely dehydrated and embedded in paraffin to prepare 4μm tissue sections for immunohistochemical staining (P-gp).

Immunohistochemical staining

Immunohistochemical staining method

Using the SP method, antigen repair was required for P-gp determination. The negative control was replaced with phosphate-buffered saline（PBS）buffer, and the remaining procedures were performed according to the manufacturers' instructions. P-gp positive control was provided by Beijing Zhongshan Biotechnology Co., LTD., and the positive control was colon cancer. Positive controls were set for each antibody. Routine dewaxing hydration was performed using xylene twice for 15 min each, hydration by gradient alcohol, PBS (pH=7.4) wash for 5 min three times, incubated in a 3% hydrogen peroxide solution at room temperature for 15 min to eliminate endogenous peroxidase. Then, the sample was washed with distilled water for 5 min three times. For antigen repair, 10 ml of ethylene diamine tetraacetic acid （EDTA）

antigen repair solution was gathered and diluted with 490 ml of tristeam water, thermal repair for 3-4 min. Finally, sample cooling to room temperature and washed in PBS for 5 min three times, sealed with normal goat serum working solution, incubated at 37℃ for 40 min. Primary antibodies were added into a wet box overnight at 4℃ rewarmed at 37℃ for 45 min, washed with PBS for 5 min three times. Labeled secondary antibodies were added with biotin drops and incubated at 37℃ for 25 min, washed in PBS for 5 min three times, incubated in horseradish peroxidase-labeled streptomycin at 37℃ for 20 min, washed with PBS for 5 min three times. The chromographic reaction was observed under a microscope in chromographic solution of 3,3-N-Diaminobenzidine Tertrahydrochloride （DAB）. The sample was rinsed with running water, re-stained gently with hematoxylin for 10-30 s, gradient dehydration with alcohol, transparent Xylene, then mounted with a neutral mounting sheet.

Evaluation of immunohistochemical staining results

DAB immunohistochemical color development system was used, and the positive results showed brown-yellow particles in the corresponding positions. P-gp positive particles were mainly distributed in the lamina propria of colonic mucosa and intestinal epithelium, and the clear brownish yellow color in the membrane and cytoplasm was positive. Ten pathological cells in the high-power field were randomly counted. P-gp treated the number of positive cells < 10% as negative, 10-25% as (+), and 25-75% as (++). This refers to the standard provided by Zhongshan Biotechnology Co., LTD.

Reverse transcription-polymerase chain reaction (RT-PCR)

Primers

Primers designed by Primer Premier 5.0 were used in reference [14]. MDRI and **β-**actin primers were synthesized by Beijing Cybersyn Bioengineering Company.

MDR1 specific primers:

Forward: 5'-AGATCAACTCGTAGGAGTGTC-3'

Reverse: 5'-GTTTCTGTATGGTACCTGCAA-3'

β-actin primers:

Forward: 5’-GAGACCTTCAACACCCCAGCC-3’

Reverse: 5’-GGCCATCTCTTGCTCGAAGTC-3’

Tissue total RNA extraction

The fresh specimens collected by colonoscopy biopsy or tissue biopsy after surgery were immediately stored in a -80℃ cryogenic refrigerator to detect the expression level of MDR1.

Trizol extraction [15]

Frozen tissue samples were harvested and ground to powder and transferred to a pre-cooled 1.5 ml EP tube before the liquid nitrogen was volatilized. LML Trizol was added, the microtissue homogenizer was inserted into the homogenizer for 2-3 min, and then chloroform was added and oscillated for 15 s and left to stand for 5 min, followed by centrifugation at 12000 *rpm* at 4℃ for 15 min. The upper water component was carefully collected into another new EP tube. Isopropanol was pre-cooled at -20 ° C equal to the supernatant was added and mixed well. After 10 min of ice bath, the supernatant was discarded and dried, added with 1 ml 75% ice ethanol and mixed well, the precipitation was washed fully, centrifuged at 8000 *rpm* at 4℃ for 5 min, then the supernatant was discarded and the precipitation was dried. For immediate use, the sample was left at an ambient temperature for 20 min and added with 30μl of DEPC water. If the sample was not used immediately, 75% ethanol LML was added and stored at -80℃.

RNA quality identification

The purity of RNA was good ($OD_{260}/OD_{280}\approx1.97$) at OD_{260} and OD_{280} in the ULTRAVIOLET spectrophotometer. The 5S, 18S and 28S bands could be seen clearly by formaldehyde gel electrophoresis, and the extracted RNA was not degraded, as shown in Figure 1.

RT - PCR

Reverse transcription and the first round OF PCR was carried out in the same centrifugation tube, and the reaction mixture consisted of 5 μl of TaqDNA polymerase buffer, 25 mmol $MgCl_2$ 3 μl, 4×10 mmol dNTP 4 μl, 10 μlmol MDRl and P-actin arch L (both composed of upstream and downstream primers), 3UL of TaqDNA polymerase 1U and 26 μl of RNA extract. Reverse transcription reaction conditions: 42℃ for 30 min. PCR reaction conditions: pre-denaturation at 94℃for 5 min. Circulating parameter: 94℃ for 1 min, 50 ℃ for 1 min and 72 ℃ for 1 min, 35 cycles and extension for 5 min.

Identification of amplified product

The 2% agar-gel (containing ethidium bromide 0.5 μl/ml) was prepared by TAE, and the 10UL amplification products were sampled for 120 mins by 100V electrophoresis. The amplification products of MDRl and B-actin were identified by the ultraviolet lamp, which were 167 bp and 301 bp, respectively, consistent with the designed amplification fragments of MDR1 and P-Actin primers.

Quantitative analysis

Cs-910 chromatographic scanner manufactured by the Shimadzu company was used to scan two bands of MDRl and B-actin primers. The length and width were 2.1 mm and 0.1 mm. Wavelength input was 550 nm. Data was inputted into the computer for relative quantitative analysis, MDR1/B-actin is the relative content of MDR1.

Statistical analysis

SPSS 19.0 statistical software package was used for statistical analyses (SPSS Inc., Chicago, IL). *Chi*-squire test was used for the comparison of percentage, and *t*-test was utilized for the comparison of measurement data between two groups. A *P* value of less than 0.05 was considered as statistical significance.

Results

Immunohistochemical staining results

P-gp positive particles were mainly distributed in the lamina propria of colonic mucosa and intestinal epithelial cells, showing a clear brownish-yellow color in the cell membrane and cytoplasm. In the control group, P-gp was positively expressed in epithelial cells and lamina propria (Figure 2A), P-gp in colonic mucosa expressed positive signal in epithelial cells and lamina propria (Figure 2B), P-gp in UC colonic mucosa expressed positive signal in both epithelial cells and lamina propria cells (Figure 2C), and P-gp of UC colonic mucosa showed positive signal in both epithelial cells and lamina propria cells (Figure 2D).

Effect of ASA on P-gp expression of in UC

In 58 UC patients treated with ASA, the expression rates of P-gp before and after treatment in the effective group of UC were statistically significant compared with those in the normal control group ($P<0.05$). However, the positive expression rates of P-gp after treatment in the effective and ineffective groups did not significantly differ compared with those before treatment (all $P>0.05$), as shown in Table 4 **and** figure 3.

Effect of glucocorticoids on P-gp expression in UC

In 53 patients receiving glucocorticoid hormones, the positive expression rate of P-gp was significantly down-regulated in the effective groups compared with that in the control group before and after treatment (both $P<0.05$). The positive expression rate of P-gp in the ineffective group did not differ from that in the control group before and after treatment (both $P>0.05$). The positive expression rate of P-gp in the ineffective group was significantly higher compared with that in the control group after treatment ($P<0.05$). In the ineffective group, the positive expression rate of P-gp was significantly increased after treatment ($P<0.05$). In the ineffective group, the positive expression rates of P-gp before and after treatment were significantly higher than those in the effective group (both $P<0.05$), as shown in Table 5 **and** figure 4.

Effect of immunosuppressive agents on P-gp expression in UC

In 37 UC cases using immunosuppressant drugs, the positive expression rates of P-gp in the effective group before treatment and after treatment were significantly decreased than those in the control group (both $P<0.05$). In the effective group, the positive expression rate of P-gp did not significantly differ before and after treatment ($P>0.05$). The positive expression rate of P-gp before treatment did not differ between

the ineffective and control groups (P>0.05), whereas it was significantly increased after treatment compared with that in the control group (P<0.05). In the effective group, the positive expression rates of P-gp did not significantly differ before and after treatment (P>0.05), as shown in Table 6 **and** figure 5.

RT-PCR of MDR1 gene

Expression of MDR1 gene as shown in Figure6.

Effect of ASA on MDR1 gene expression in UC
In 58 UC patients with UC treated with ASA, the expression levels of MDR1 gene before and after treatment in the effective group were 0.590±0.071 and 0.514±0.018, significantly lower compared with 1.374±0.022 in the control group (both P<0.05). However, the expression level of MDR1 gene after treatment in the effective and ineffective groups did not significantly differ from those before treatment (all P>0.05), as shown in Table 7 and Figure 7.

Effect of glucocorticoid on MDR1 gene expression in UC

In the effective group, the expression levels of MDR1 gene before and after treatment were 0.675±0.103 and 0.509±0.106, significantly lower than 1.374±0.022 in the control group (both P<0.05). In the effective group, the expression level of MDR1 gene did not significantly differ before and after treatment (P>0.05). The expression level of MDR1 before treatment in the ineffective group did not significantly differ from that in the control group (P>0.05), and the expression level was considerably up-regulated after treatment (P<0.05). In the ineffective group, the expression levels of MDR1 gene before and after treatment were significantly higher than those in the effective group (both P<0.05), as shown in Table 8 **and** Figure 8.

Effect of immunosuppressive agents on MDR1 gene expression in UC

In the effective group, the expression levels of MDR1 gene before and after treatment were 0.618±0.095 and 0.523±0.0201, which significantly differed from 1.374±0.022 in the control group (both P<0.05). The expression levels of MDR1 gene did not significantly differ before and after treatment in the effective group (P>0.05). In the ineffective group, the expression level before treatment did not significantly differ compared to that in the control group (P>0.05), whereas it was dramatically up-regualted post-treatment (P<0.05). The expression levels of MDR1 gene before and after treatment in the ineffective group were statistically significant compared with those in the effective group (both P<0.05), as shown in Table 9 **and** Figure 9.

Discussion

Immunohistochemical staining and RT-PCR detection of MDR1 gene showed that the expression rates or levels of P-gp and MDRl genes in the effective group of amino salicylic acid drugs before and after treatment and the ineffective group before and after treatment were significantly different from those in the normal control group. However, there was no statistical difference in the expression rate or expression

amount of P-gp in the effective and ineffective groups after treatment compared with before treatment. This indicates that the internal MDR-gene expression in patients with ulcerative colitis has been changed before the treatment, and drug treatment does not affect MDR-gene expression, that is, it does not change the increase of MDR-gene resistance, nor does it reduce the expression.

Salazosulfapyridine is a kind of nF-KB (P65) inhibitor, which exerts potent inhibitory effect on the activation of nF-KB (P65). Uetsuka H has investigated the effect of 5-fluorouracil (5 Fu) and willow nitrogen sulfanilamide pyridine on pancreatic cancer cell apoptosis and proliferation *in vitro* and *in vivo* in different concentrations of nitrogen sulfanilamide pyridine with the extension of time for cell growth inhibition rate in a declining trend, but a dose-response relationship exists with the increase of the concentration of nitrogen, sulfanilamide apoptosis induced by pyridine is basically unaffected, but have it has effect on the cell cycle as the Go/G1 cells proportion increases gradually and the proportion of S phase decreases significantly. This may be associated with the inhibition of NF-KB(P65) activation by salazine sulfapyridine and the difficulty in initiating the transcription target gene Cyclin D1, resulting in the hindrance of the Go/G1 phase to S phase transformation. Salazosulfapyridine blocking NF-xB(P65) activation has an inhibitory effect on cell proliferation. Salazosulfamidine inhibites the proliferation of pancreatic cancer cells and enhances 5-FU-induced apoptosis. The synergistic growth inhibition effect of the two is closely related to the synergistic induction of apoptosis and cell cycle arrest in Go/G1 phase. The down-regulation of mRNA and protein levels of Bcl-2, Cyclin D1, MDRl and NF-KB(P65) is one of the reasons for the synergistic enhancement of 5-Fu-induced apoptosis of bXPC-3 cells and the reduction of MDR gene expression in pancreatic cancer [16]. Salazosulfapyridine can synergistically enhance the apoptosis of 5-Fu-induced bXPC-3 apoptosis in nude mice with pancreatic cancer. The up-regulated Bax level and down-regulated bcl-2, cyclinD1, MDR1 and NF-KB(P65) levels may be one of the mechanisms of bXPC-3 apoptosis in subcutaneous heterotopic xenograft cells of pancreatic cancer induced by combining the action of salazine and 5-Fu. Although the literature suggests that willow nitrogen sulfanilamide pyridine can coordinate 5-FU to indirectly down-regulate the MDR1 mRNA level, but our research shows that there was no significant change in the expression of MDR1 willow nitrogen sulfanilamide pyridine, probably because that the amino acid drugs, such as willow nitrogen sulfanilamide, does not alter the MDR1 gene, and the indirect effect is extreamely weak concealed cut MDR1 gene level. Therefore, MDR1 gene and its expression product P-gp do not significantly change in UC patients.

There was no statistically significant difference in the positive expression rate or expression level of the ineffective group before treatment compared with that of the control group, while the positive expression rate or expression level in the ineffective group after treatment was significantly increased compared with that in the control group, and the reaching rate or expression level of MDR1 gene and P-gp in the ineffective group after treatment were significantly increased compared with that before treatment. The positive expression levels or rates of MDR1 gene and P-gp in the ineffective group were significantly higher than those in the effective group. These results are consistent with the notion that troche against plug with platelet activation in refractory UC patients, compared with the controls, the serum level of P-gp

is significantly elevated in refractory UC patients because the sugar cortical hormone is the substrate of P-gp. There is no correlation between effective drug treatment of patients with UC with MDR1 expression.

The therapeutic effect of UC largely depends on the patient's response to glucocorticoid therapy. For some patients, there is still no response after sufficient application, known as drug resistance, thus causing treatment delay [17]. Hormone treatment failure of UC patients significantly increases the MDR1 expression of peripheral blood lymphocytes, and MDR1 gene in peripheral blood lymphocytes and intestinal mucosa cells is the only P-gp expressed genes, and intestinal mucosa cells and peripheral blood lymphocytes of MDR1 base for expression in response to the hormone therapy play an important role in refractory UC, it is reasonable to blame MDR1 expression of P-gp. P-gp can mediate glucocorticoid resistance through multiple pathways. Firstly, P-gp can induce glucocorticoid resistance by acting on the drug transport pump and protecting cells from apoptosis. In addition, P-gp can also mediate hormone resistance by acting in the overall cellular immune system, such as acting on immune cells and certain aspects of cytokines' effects. The peripheral blood mononuclear cells of 10 healthy people are stimulated with plant hemagglutinin, and P-gp monoclonal antibody is employed to block the work energy of P-gp [18]. Flow cytometry is employed to detect the levels of interleukin-2、4、6、10（IL-2, 4, 6, 10）, interferon-gamma（IFN-γ）, tumor necrosis factor-α（TNF-α） in the cultured supernatant, revealing that P-gp MAb could inhibit the release of cytokines, such as IL-2, 4, IFN-γ and TNF-α.

The expression levels of P-gp in peripheral lymphocytes and intestinal mucosal epithelial cells of patients with UC were significantly higher than those of drug-sensitive patients and the controls, suggesting that the overexpression of P-gp, the product of MDR1, may be one of the causes of hormonal therapy failure in patients with UC. In UC patients without hormone treatment, the expression and activity of P-gp in peripheral blood, intestinal mucosa lamella propria and intestinal epithelial lymphocytes are significantly lower than those in the normal controls [19]. However, the expression levels of P-gp in the peripheral blood mononuclear cells and MDR1 mRNA level do not significantly differ between healthy subjects and patients treated with hormone therapy, whereas the values are significantly higher than patients without hormone therapy. For those patients treated with hormone treatment with peripheral blood monocytes MDR1mRNA expression quantity and the total dosage of hormone shows a significant positive correlation, indicating that the high expression of MDR1mRNA in UC patients is a response induced by the application of high-dose hormone therapy [20].

Comparison of the expression levels or rates of MDR1 gene and P-gp among three groups before and after corresponding treatment demonstrate that both hormones and immunosuppressants can up-regulate P-gp expression to produce acquired MDR1, and the mechanism of action may be that lymphocytes, as a protective mechanism, can specifically increase P-gp expression and reduce the toxic effect of drugs stimulated by external stimuli. Fiedler also found that glucocorticoid and spore fungal element such as joint intervention is also found in rat liver and intestinal MDR genes, and P-gp protein expression were significantly elevated, multi-resistant genes and P-gp protein expression is not only related to individual genetics, glucocorticoid and ring spore fungus itself can raise MDR genes and P-gp

protein expression [21]. Therefore, although competitive substrate drug resistance reversals can increase drug efficacy in a short period of time, the higher expression of MDR-induced genes and P-gp proteins may be related to drug resistance or drug dependence after long-term use, which may also be an important reason for the recurrence and inefficiency of multiple diseases. For MDR genes and P-gp protein expression and high disease activity of lupus patients with oral hormone can show the hormone resistance alone, but immunosuppressive agents such as cyclophosphamide and glucocorticoids methylprednisolone can significantly reduce the joint impact of $CD4^+$, $CD8^+$, $CD19^+$ cells that increase MDR genes and the expression of P-gp proteins, hence the disease activity can be significantly controlled, the counterparts and the sensitivity of glucocorticoids restored. Therefore, lowering the P-gp expression through immunosuppressive agents and glucocorticoids is an important measure to overcome hormone resistance [22]. P-gp may be an important factor affecting the clinical efficacy of hormones in patients with systemic lupus erythematosus. Different expression levels of P-gp in lymphocytes of patients with systemic lupus erythematosus indicate different hormone requirements [23], and the combination of P-gp and immunosuppressive agents may yield satisfactory therapeutic results. However, it is necessary to precisely determine the dose of type or dose of aminosalicylic acid / steroids that is required for the influence of the expression level or localization of MDR1in clinical settings.

Conclusions

The findings in the present study demonstrate that ASA exerts no significant effect upon the expression levels of MDR gene in UC patients. Nevertheless, administration of glucocorticoids and immunosuppressive agents can significantly up-regulate the expression levels of MDR gene in UC patients, whereas the underlying mechanism remains to be further elucidated.

Declarations

Availability of data and materials

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

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Contributions

Conception and design：Y. Chen ,P. Wang, Y. J. Zhang .Analysis and interpretation of data: Y. Chen ,P. Wang, Y.J. Zhang .Drafting of the article:Y. Chen , Y.J. Zhang .Critical revision of the article for important intellectual content: Y. Chen .,P. Wang , Y. Zhang,X.Y. Du, and Y. J. Zhang .Final approval of the article:Y. Chen ,P. Wang , Y. Zhang,X.Y.Du, and Y.J. Zhang . Statistical expertise: P. Wang；Y. Chen, Y. J. Zhang . Administrative, technical, or logistic support: Y. Chen , P. Wang .Collection and assembly of data: Y. Chen , P. Wang. Obtaining funding： Y.J. Zhang. Study supervision：Y.J. Zhang.

Ethics approval and consent to participate

This study was approved by the ethics committee of the first affiliated hospital, and college of clinical medicine of henan university of science and technology (ethics number: 2014-03-B034). Informed consent for all patients：including the patients of the control group： was obtained prior to therapy and performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Comparison of general data

Grouping	Case number (*n*)	gender（*n*,%）		age（years,`*X*±*S*）
		male	female	
experimental group	148	83(56.08%)	65(43.92%)	42.40±13.21
Control group	45	25(55.56%)	20(44.44%)	36.70±11.41
c^2/*F* value		1.213		1.737
P value		0.749		0.165

Table 2 Truelove-Witts Severity Index for UC

Item	Mild	Severe
Excrement (time/d)	<4	>6
Hematochezia	light or no	severe
Body temperature (℃	normal	>37.5
Pulse (time/min)	normal	>90
Hemoglobin	normal	>75%
Erythrocyte sedimentation rate (mm/1 h)	<30	>30

Table 3 Sutherland disease activity index

Item	Scoring			
	0	1	2	3
Diarrhea	Normal	1-2 time/d	3-4 times/d	5-6 times/d
hemafecia	No	A little	Obvious	Give priority to with blood
Mucous membrane performance	Normal	Mildly brittle	moderately brittle	Severely brittle with exudation
The physician evaluates the condition	Normal	Mild	Moderate	Severe

Note: The total score below 2 is classified as symptom remission; those between 3-5 are classified as mild activity; 6-10 as moderate activity; and 11-12 as heavy activity.

Table 4 Effect of ASA drugs on the expression of P-gp in UC

Group	Total (n)	Positive(n)	Negative(n)	Positive rate (%)
Control group	45	14	31	31.1
Effective group				
pre-treatment	44	6	38	13.6[a]
post-treatment	44	8	36	18.2[a,b]
Ineffective group				
pre-treatment	14	2	12	14.3[a]
post-treatment	14	2	12	14.3[a,b]

Note: [a] $P<0.05$ vs normal control; [b] $P>0.05$ vs pre-treatment

Table 5 Effect of glucocorticoid on P-gp expression in UC

Group	Total (n)	Positive(n)	Negative(n)	Positive rate (%)
Control group	45	14	31	31.1
Effective group				
pre-treatment	42	8	34	19.0[a]
post-treatment	42	7	35	16.7[a,b]
Ineffective group				
pre-treatment	11	3	8	27.2[a]
post-treatment	11	5	6	45.4[a,b]

Note: [a]*P*<0.05 vs control group; [b]*P*>0.05 vs pre-treatment; [c]*P*<0.05 vs pre-treatment; [d]*P*<0.05 vs effective group before treatment, [c]*P*<0.05 vs effective group post-treatment.

Table 6 Effect of immunosuppressants on P-gp expression in UC

Group	Total (n)	Positive(n)	Negative(n)	Positive rate (%)
Control group	45	14	31	31.1
Effective group				
pre-treatment	28	5	23	23.9[a]
post-treatment	28	6	22	19.8[ab]
Ineffective group				
pre-treatment	9	3	6	33.3[d]
post-treaent	9	4	5	44.4[abe]

Note: [a]*P*<0.05 vs normal control; [b]*P*>0.05 vs pre-treatment; [c]*P*<0.05 vs pre-treatment; [d]*P*<0.05 vs effective group pre-treatment; [e]*P*<0.05 vs effective group post-treatment

Table 7 Effect of ASA drugs on the expression level of MDR1 gene in UC (mean±SD)

Group	Total (n)	Relative expression of MDR1
Control group	45	1.374±0.022
Effective group		
pre-treatment	44	0.590±0.071[a]
Post-treatment	44	0.514±0.018[ab]
Ineffective group		
pre-treatment	14	0.454±0.073[a]
Post-treatment	14	0.314±0.062[a]

Note: [a]*P*<0.05 vs normal control; [b]*P*>0.05 vs pre-treatment

Table 8 Effect of glucocorticoid on the expression level of MDR1 gene in UC

Group	Total (n)	Relative expression of MDR1
Control group	45	1.374±0.022
Effective group		
pre-treatment	42	0.675±0.103[a]
post-treatment	42	0.509±0.106[ab]
Ineffective group		
pre-treatment	11	1.249±0.073[d]
post-treatment	11	1.867±0.151[abe]

Note: [a]*P*<0.05 vs control group; [b]*P*>0.05 vs pre-treatment; [c]*P*<0.05 vs pre-treatment; [d]*P*<0.05 vs effective group pre-treatment; [e]*P*<0.05 vs effective group post-treatment

Table 9 Effect of immunosuppressive agents on the expression of MDR1 gene in UC (mean±SD)

Group	Total (n)	Relative expression of MDR1
Control group	45	1.374±0.022
Effective group		
pre-treatment	28	0.618±0.095[a]
post-treatment	28	0.523±0.0201[a,b]
Ineffective group		
pre-treatment	9	1.263±0.0833[d]
post-treatment	9	1.885±0.054[a,b,e]

Note: [a]*P*<0.05 vs control group; [b]*P*>0.05 vs pre-treatment; [c]*P*<0.05 vs pre-treatment; [d]*P*<0.05 vs effective group pre-treatment; [e]*P*<0.05 vs effective group post-treatment.

Figures

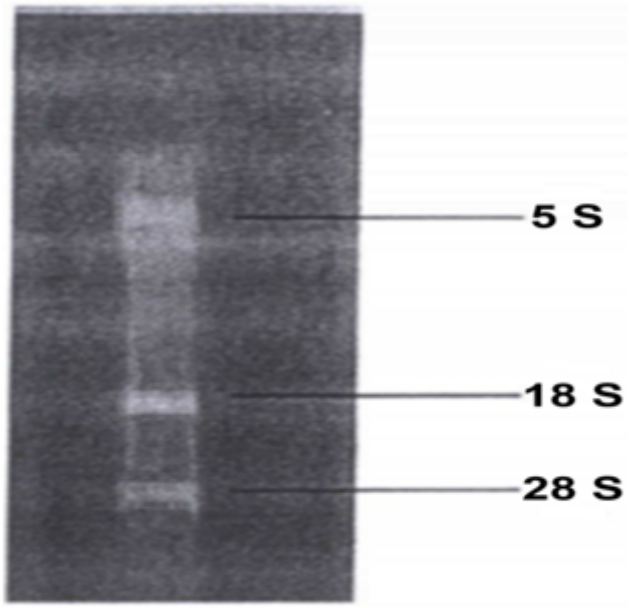

Figure 1

Total RNA integrity test

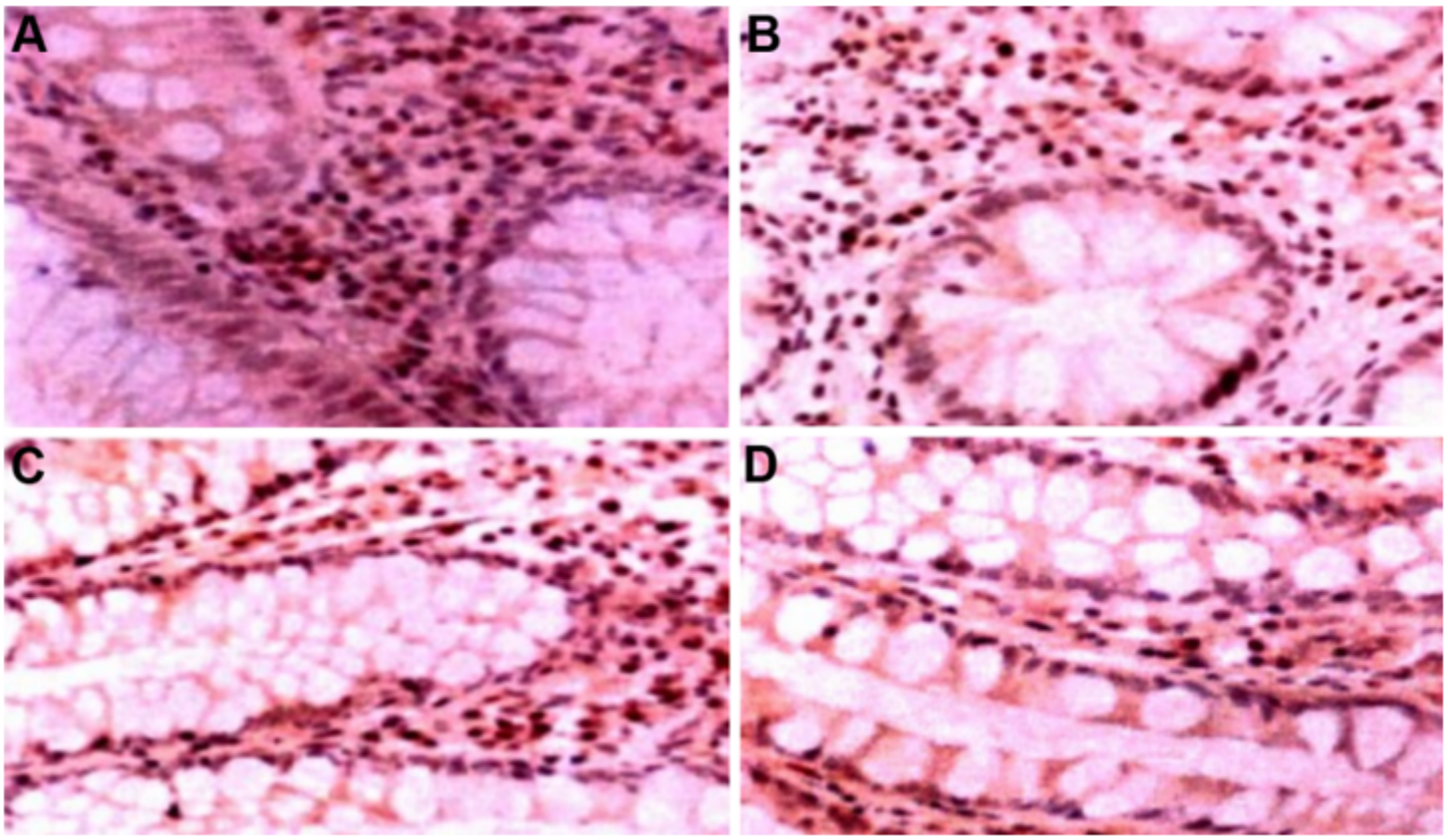

Figure 2

Detection results of immunohistochemical staining of P-gp (SP×400)

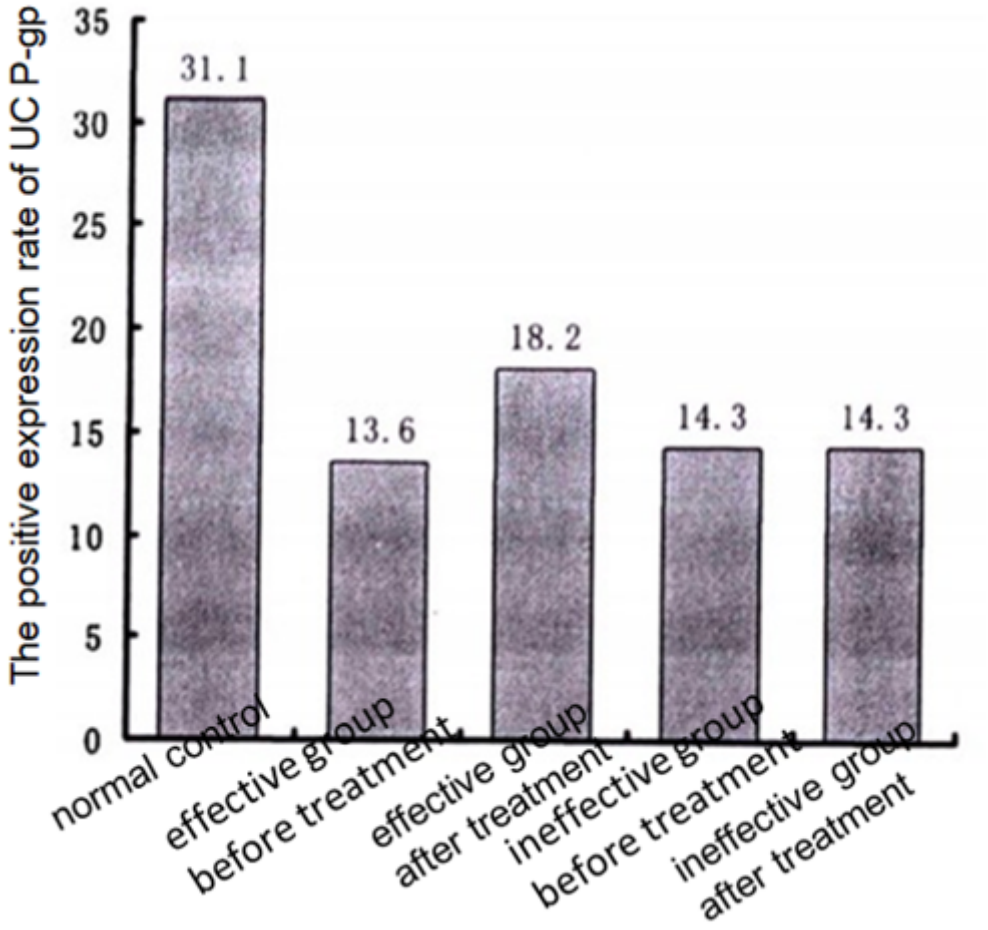

Figure 3

The effect of aminosalicylic acid drugs on the expression of P-gp in ulcerative colitis

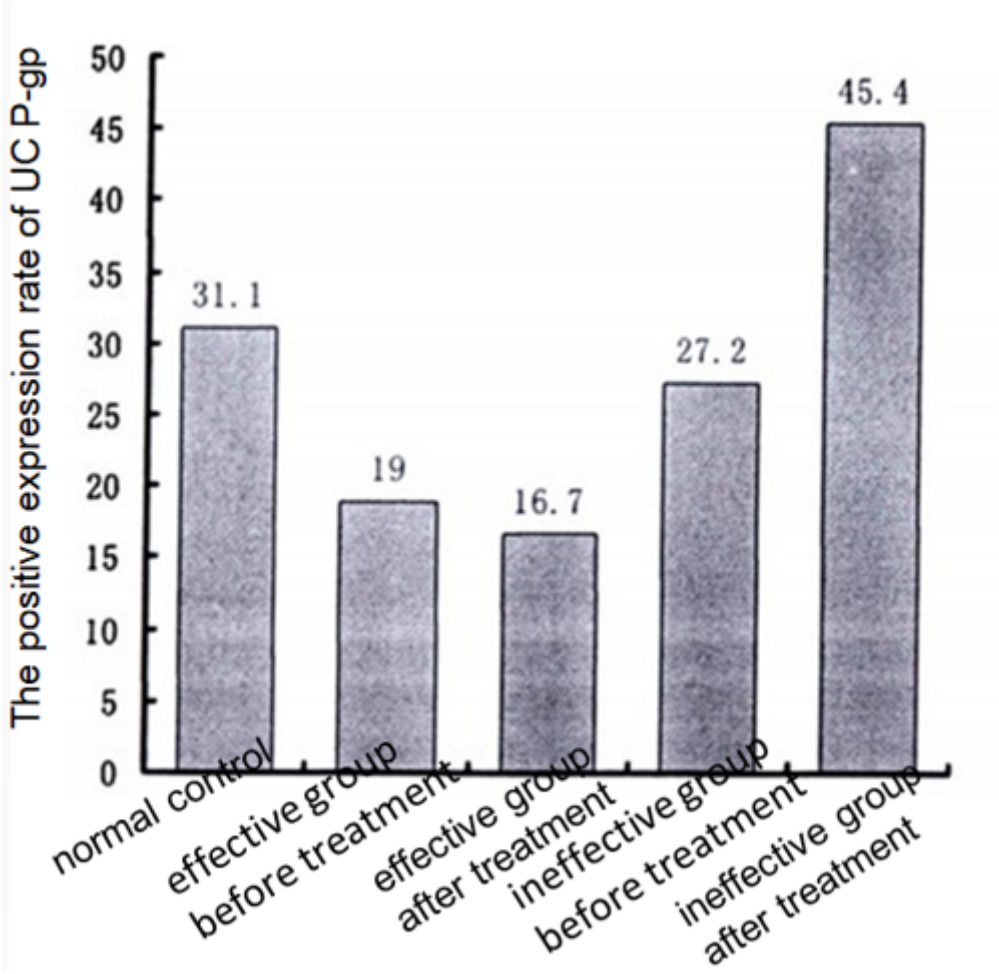

Figure 4

The effect of glucocorticoid on P-gp expression in ulcerative colitis

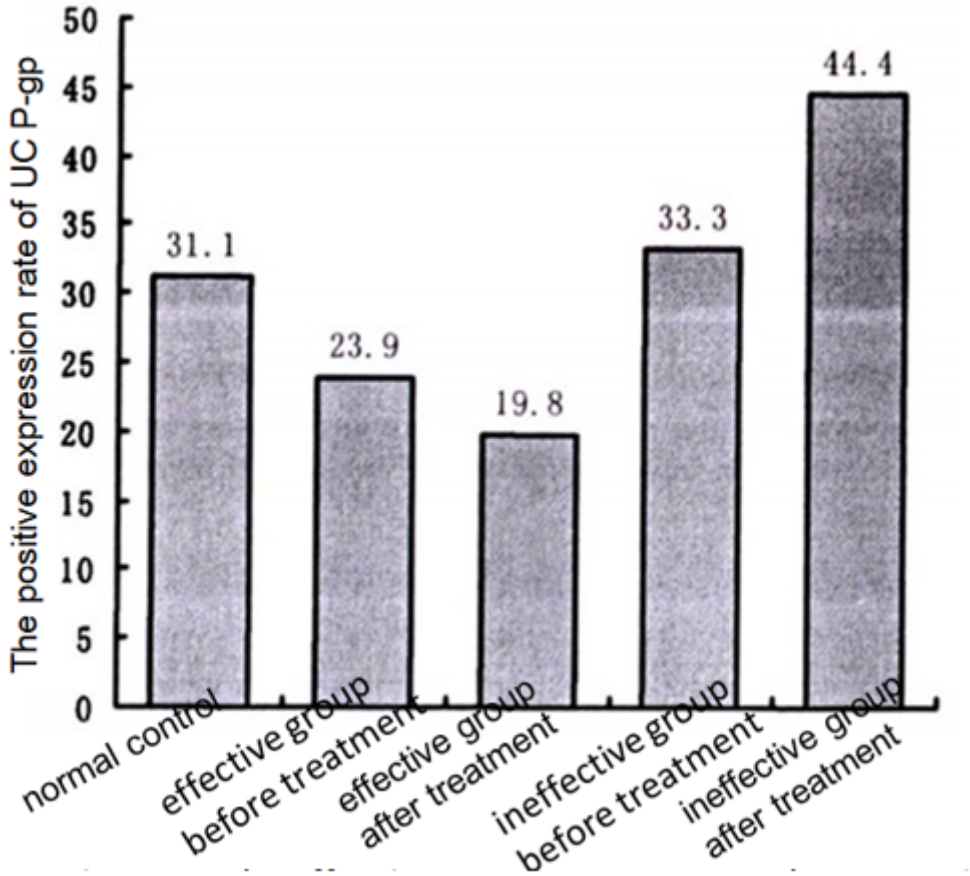

Figure 5

The effect immunosuppressant on the expression of P-gp in ulcerative colitis

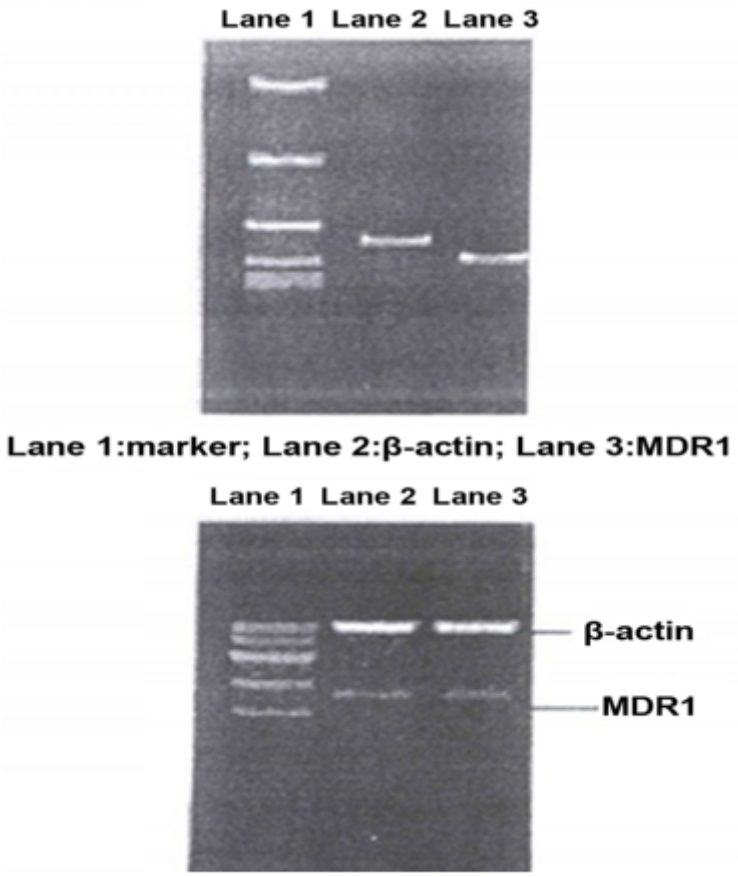

Figure 6

RT-PCR of MDR1 gene. Lane 1: marker; Lane 2: MDR1gene in the control group; Lane 3: MDR1 gene expression in the UC group

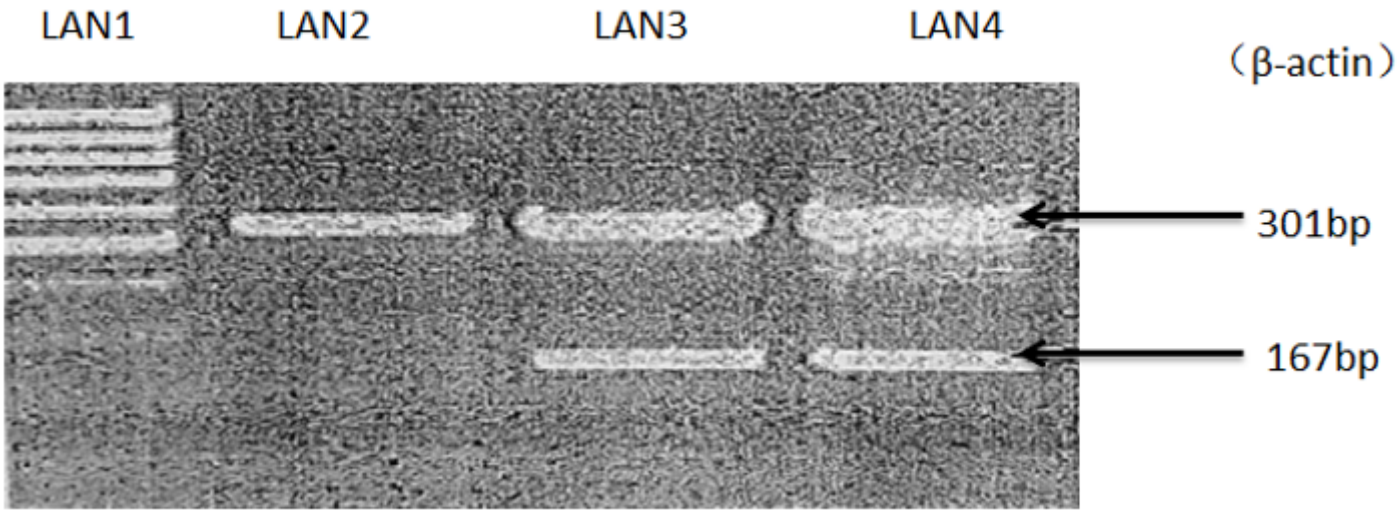

Figure 7

Expression of MDR1 gene in each group LAN1: marker; LAN2: MDR1 was negatively expressed in the group treated with ASA; LAN3: MDR1 was positively expressed in the group treated with ASA; LAN4:

MDR1 was positively expressed in normal colon tissue.

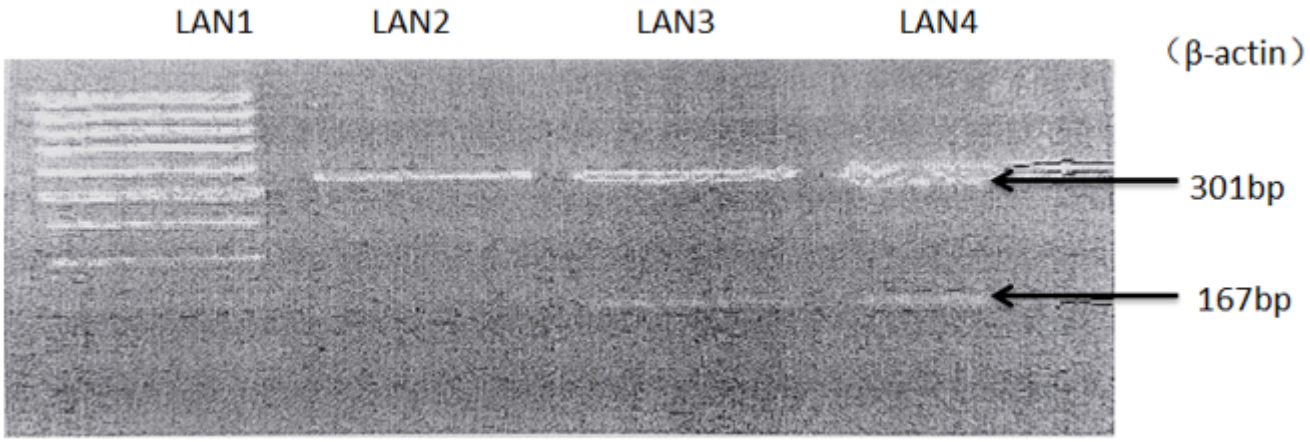

Figure 8

Expression of MDR1 gene in each group LAN1: marker; LAN2: MDR1 was negatively expressed in the group treated with glucocorticoid; LAN3: MDR1 was positively expressed in the group treated with glucocorticoid; LAN4: MDR1 was positively expressed in normal colon tissue.

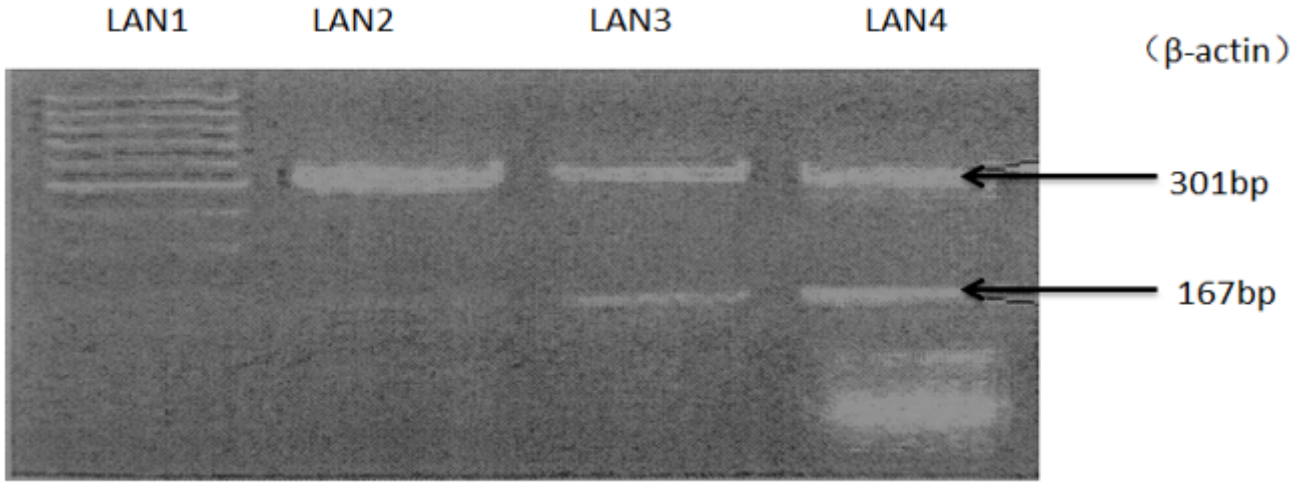

Figure 9

Expression of MDR1 gene in each group LAN1: marker; LAN2: MDR1 was negatively expressed in the group treated with immunosuppressive; LAN3: MDR1 was positively expressed in the group treated with immunosuppressive; LAN4: MDR1 was positively expressed in normal colon tissue.