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Multidrug resistance gene and its relationship to ulcerative colitis and immune status of ulcerative colitis

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ABSTRACT. We examined the relationship among the multidrug resistance (*MDR1*) gene product P-glycoprotein (P-gp), ulcerative colitis, and immune status under ulcerative colitis. MDR1 P-gp expression and interleukin-8 levels in ulcerative colitis were determined using immunohistochemistry and a double-antibody sandwich avidin-biotin complex-enzyme-linked immunosorbent assay, respectively. Nitric oxide content and nitric oxide synthase activity in the colonic mucosa were determined using a colorimetric method; $CD4^+$ and $CD25^+$ T cell subset percentages in the peripheral blood were determined by flow cytometry. The positive expression rate of P-gp in patients with ulcerative colitis (17.4%) was significantly lower than that in the control group (31.4%). The expression rate decreased to 10.1, 9.2, and 8.3% after 12, 18, and 24 months of treatment, respectively, which were significantly lower than the expression rate before treatment (17.4%). P-gp expression levels during the remission phase and active phase

of ulcerative colitis were 15.2 and 17.1%, respectively, which were significantly lower than that in normal controls (31.4%). Compared with P-gp-negative patients, nitric oxide content, nitric oxide synthase activity, and interleukin-8 levels were significantly higher in P-gp-positive patients with moderately active, severely active, early onset, chronic relapsing, chronic persistent, and acute fulminant ulcerative colitis. $CD4^+$ and $CD25^+$ T cell subsets were significantly lower in the peripheral blood of patients with severely active and acute fulminant ulcerative colitis than in control subjects. Expression of the multidrug resistance gene and its product P-gp was observed in normal colon tissues and may be closely related to ulcerative colitis.

Key words: Colitis; Drug resistance; Immune status; NOS activity; IL-8; $CD4^+$ and $CD25^+$ T cells

INTRODUCTION

Recent studies have indicated that the multidrug resistance gene (*MDR1*) expression product, P-glycoprotein (P-gp), is related to the pathogenesis of ulcerative colitis (UC) (Mendoza et al., 2007). Potocnik et al. (2004) suggested that P-gp expression and activity are lower in the peripheral blood, colonic tissue lamina propria, and colonic intraepithelial lymphocytes in UC patients compared to control patients. Farrell and Kelleher (2003) demonstrated that inflammatory bowel diseases, including Crohn's disease and UC, are resistant to glucocorticoid and immunosuppressant drugs, and that patients requiring surgical treatment have higher P-gp levels in peripheral lymphocytes and the colonic epithelium compared to drug-sensitive and normal control groups. These results differ from those reported by Potocnik et al. (2004).

Studies have focused on the pathogenesis and immunological mechanisms of UC. Studies examining the histopathology and clinical characteristics of UC and the effectiveness of immunosuppressive therapy indicate that the immune system plays an important role in UC development (Spahn and Kucharzlk, 2004).

The immune system is an expansive network that requires coordination and interaction between its various parts to maintain the body immune response and immune tolerance. Disruption of any part of the immune system can lead to an immune imbalance (Forchielli and Walker, 2005). An abnormal response of the body immune system to normal intestinal flora plays a major role in the pathogenesis of UC, which involves the entire immune response (Thompson-Chagoyán et al., 2005). Multidrug resistant (*MDR1a*) genes are expressed on both immune and non-immune cell surfaces and have multiple functions (Chandran et al., 2003). Dommels et al. (2007), using FVB wild-type and FVB multi-drug resistance gene knockout (*MDR1a*$^{-/-}$) mice, observed P-gp in colitis and determined the humoral and cellular immune function in a UC group and non-UC group of *MDR1a*$^{-/-}$ mice. A study of the clinical features, pathology, and immunohistochemistry of UC in 124 *MDR1a*$^{-/-}$ mice demonstrated that 24 mice (21%) had diarrhea, weight loss, and similar pathology, which was similar to the symptoms observed in humans with UC, as well as $CD4^+$ T lymphocyte and B lymphocyte infiltration in the colon mucosa lamina propria. Serum antibody and cytotoxic T lymphocyte function in *MDR1a*$^{-/-}$ mice without UC were similar to those in wild-type FVB mice. These results indicate that colitis can occur in mice lacking *MDR1a*; however, the pathogenesis of

colitis is unrelated to the systemic immune response, and is possibly due to an abnormal local colonic epithelial cell barrier. Asari et al. (2010) confirmed that *MDR1a* is the only P-gp gene expressed in mouse intestinal epithelial cells and intestinal lymphocytes and established an *MDR1a*$^{-/-}$ mouse model of inflammatory bowel disease.

In this study, we used immunohistochemical methods to detect the UC *MDR1* gene product P-gp, the double-antibody sandwich avidin-biotin complex-enzyme-linked immunosorbent assay (ELISA) method to measure serum interleukin (IL)-8 levels, colorimetry to measure nitric oxide (NO) content and nitric oxide synthase (NOS) activity in the colonic mucosa, and flow cytometry to determine peripheral blood $CD4^+$ and $CD25^+$ T cell subset percentages. The purpose of this study was to determine the relationship between the *MDR1* product P-gp and the clinical characteristics of UC in humans. We also examined the relationship with the immune status of UC patients to provide a theoretical and practical basis for preventing and managing UC.

MATERIAL AND METHODS

Patients and controls

Patients were selected from outpatients and inpatients treated in the 3 affiliated hospitals of Henan University of Technology, China, from March 2007 to December 2012. Of the 109 patients, 68 were male and 41 were female aged 17-70 years, with an average age of 41.2 years. In the control group, electronic colonoscopy samples were acquired from 15 patients without intestinal symptoms who were confirmed to have normal pathology, no history of irritable bowel syndrome, past history of UC, and no drug allergies. Another 20 normal surgical samples of bowel tissue were obtained from patients without inflammatory bowel disease. Of these 35 samples, 19 were from males and 16 were from females aged 19-68 years, with an average age of 39.4 years. Study subjects were followed-up for 24 months with a starting time indicated by the treatment time or discharge time. The last patient included was in December 2010 and was followed-up until December 2012. Patients were divided into groups, including a no-drug group and groups that had been treated with oral aminosalicylate drugs, oral or intravenous corticosteroids, oral or intravenous corticosteroids and immunosuppressive agents, topical application of sulfasalazine, 5-aminosalicylic acid, glucocorticoids, and traditional Chinese medicine.

The study protocol was approved by the Ethics Committee of Henan University of Technology. Informed consent to participate in the study and undergo surgery was obtained from all patients.

Pathological diagnoses

All endoscopic biopsy and resection specimens were evaluated by at least 2 experienced pathologists. In cases where there was a disagreement, a third pathologist reviewed the case and gave a final diagnosis.

Clinical severity classifications

In accordance with the UC treatment guidelines of the American Gastroenterological Association (Arnold et al., 1998), disease severity was graded as mild, moderate, or severe.

Endoscopic grading standards

According to Baron's improved endoscopic grading standards of UC (Saverymuttu et al., 1986), the following grades were used: grade 0, grade I, grade II, grade III, and grade IV.

Immunohistochemical staining

The streptavidin-peroxidase method was used and antigen retrieval was required before P-gp measurement. As a negative control, normal serum was used rather than primary antibody. The remaining steps were the same. The P-gp-positive control was the serum of a colon cancer patient (Figures 1 and 2).

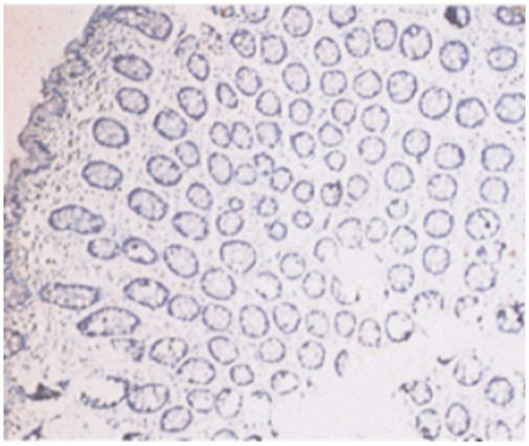

Figure 1. Negative expression of P-gp in colonic mucosa. SP method 400X.

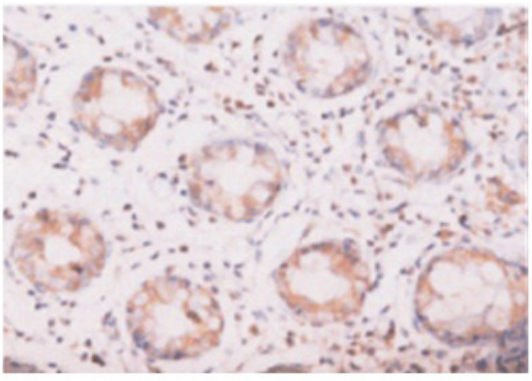

Figure 2. Positive expression of P-gp in the interstitial lymphocyte membrane and nucleus of colonic epithelial cells. P-gp-positive particles were mainly located in the lamina propria of the colonic mucosa and intestinal epithelial tissue and are shown as clear brown color in the cell membrane and cytoplasm. SP method 400X.

Determination of serum IL-8 levels

The double antibody sandwich avidin-biotin complex ELISA was used to determine serum IL-8 levels.

NO content and NOS activity in colonic mucosa

Serum levels of NO were measured using the nitrate reductase assay. NOS activity was assessed by measuring the amount of NO from L-arginine catalyzed by NOS per unit time. By measuring total NOS activity and inducible NOS (iNOS) activity without $CaCl_2$ in the reaction solution, the Ca^{2+}-dependent catalytic arginine and structural activity was calculated by subtracting iNOS activity from total NOS activity. The Biuret method was used to determine total protein content in the homogenates. NO concentration was measured as μmol/g and NOS activity as U/mg.

Peripheral blood $CD4^+$ and $CD25^+$ T cell subsets

Flow cytometry was used to determine peripheral blood $CD4^+$ and $CD25^+$ T cell subsets.

Statistics

The SPSS 10.0 statistical package was used for data entry and statistical analysis (SPSS, Inc.; Chicago, IL, USA). Count data are reported as a percentage, and 2 sets of data were compared using the Student *t*-test. The P-gp-positive expression rate in each group was compared using the χ^2 test. A P value of <0.05 was considered to be significantly different between 2 groups.

RESULTS

P-gp and duration of UC

There were 109 cases of UC before and after treatment and 35 normal controls included in this study. Compared to the control group, the P-gp-positive rate was significantly lower in patients with UC before and after 6, 12, 18, and 24 months of treatment ($P < 0.05$). In UC patients, the P-gp-positive rate was significantly lower after 12, 18, and 24 months of treatment ($P < 0.05$); however, there was no significant change after 6 months of treatment ($P > 0.05$) (Table 1).

Table 1. P-gp and duration of ulcerative colitis (UC).

Duration of UC	N	Positive (N)	Negative (N)	Positive rate (%)
Control	35	11	24	31.4
Before treatment	109	19	90	17.4[a]
After treatment				
6 months	109	18	91	16.5[ac]
12 months	109	11	98	10.1[abd]
18 months	109	10	99	9.2[abd]
24 months	109	9	100	8.3[abd]

[a]$P < 0.05$ *vs* control; [b]$P < 0.05$ *vs* before treatment; [c]$P > 0.05$ *vs* before treatment; [d]$P < 0.05$ *vs* after 6 months of treatment.

P-gp and clinical type of UC

The clinical types of UC were divided into early onset, chronic relapsing, chronic persistent, and acute fulminant. Positive P-gp expression in all clinical types was significantly lower than in control subjects ($P < 0.05$); there was no significant difference between the early onset type and chronic relapsing or chronic persistent types ($P > 0.05$). The P-gp-positive expression rate in the acute fulminant type was significantly lower than that in the early onset type ($P < 0.05$) (Table 2).

Table 2. P-gp and clinical type of ulcerative colitis.

Clinical type	N	Positive (N)	Negative (N)	Positive rate (%)
Control	35	11	24	31.4
Early onset	37	6	31	16.2[a]
Chronic relapsing	33	5	28	15.2[ab]
Chronic persistent	24	4	20	16.7[ab]
Acute fulminant	15	1	14	6.7[ac]

[a]$P < 0.05$ *vs* control; [b]$P > 0.05$ *vs* early onset; [c]$P < 0.05$ *vs* early onset.

Disease activity in 76 cases was divided into mild, moderate, and severe. The P-gp expression rate in mild, moderate, and severe disease was significantly different from that in controls ($P < 0.05$). No significant difference in the P-gp expression rate was observed in moderate and severe disease compared to that in mild disease ($P > 0.05$) (Table 3).

Table 3. P-gp and the severity of active ulcerative colitis.

Severity	N	Positive (N)	Negative (N)	Positive rate (%)
Control	35	11	24	31.4
Active mild	40	6	34	15.0[a]
Active moderate	24	3	21	12.5[ab]
Active severe	12	2	10	16.7[ab]

[a]$P < 0.05$ *vs* control; [b]$P > 0.05$ *vs* active mild.

P-gp and the severity of UC

P-gp and clinical stages

According to the clinical stage, UC cases were classified as being in remission or being active. The P-gp-positive expression rate in the remission and active stage was significantly lower than that in controls ($P < 0.05$); however, no difference was observed between the active and remission stages ($P < 0.05$) (Table 4).

Table 4. Relationship between P-gp and clinical stage.

Stage	N	Positive (N)	Negative (N)	Positive rate (%)
Control	35	11	24	31.4
Remission	33	5	28	15.2[a]
Active	76	13	63	17.1[ab]

[a]$P < 0.05$ *vs* control; [b]$P > 0.05$ *vs* remission.

Relationship between NO content, NOS activity, and P-gp in colonic mucosa

Compared with normal controls, NO content and NOS activity in the colonic mucosa were significantly increased in active mild, moderate, and severe disease, as well as in early onset, chronic relapsing, chronic persistent, and acute fulminant disease ($P < 0.05$). NO content and NOS activity in colonic mucosa increased significantly in active *vs* remission cases, moderate and severe *vs* mild cases, and in acute fulminant *vs* early onset cases ($P < 0.05$). NO content and NOS activity increased significantly in active moderate, active severe, early onset, chronic relapsing, chronic persistent, and acute fulminant disease in P-gp-positive patients compared with P-gp-negative patients ($P < 0.05$) (Tables 5 and 6, Figures 3 and 4).

Table 5. Comparison of NO content in colonic mucosa (means ± SD) (μmol/g).

Group	N	P-gp-positive	P-gp-negative
Control	35	14.71 ± 3.29	12.65 ± 2.52
Clinical stage			
Remission	33	19.52 ± 5.83	15.27 ± 3.96
Active	76	37.14 ± 4.69[ab]	26.28 ± 4.92[abf]
Severity			
Mild	40	25.73 ± 6.28[a]	22.28 ± 5.14[a]
Moderate	24	41.16 ± 5.19[ac]	31.93 ± 4.74[acf]
Severe	12	47.53 ± 9.82[ac]	35.28 ± 9.15[acf]
Clinical type			
Early onset	37	32.19 ± 2.74[a]	22.19 ± 2.74[af]
Chronic relapsing	33	36.52 ± 8.35[ae]	25.69 ± 6.52[aef]
Chronic persistent	24	31.52 ± 4.61[ae]	21.63 ± 5.37[aef]
Acute fulminant	15	56.17 ± 8.72[ad]	41.29 ± 9.44[adf]

[a]$P < 0.05$ *vs* control; [b]$P < 0.05$ *vs* remission; [c]$P < 0.05$ *vs* mild; [d]$P < 0.05$ *vs* early onset; [e]$P > 0.05$ *vs* early onset; [f]$P < 0.05$ *vs* P-gp-positive.

Table 6. Comparison of NOS activity in colonic mucosa (means ± SD) (U/mg).

Group	N	P-gp-positive	P-gp-negative
Control	35	8.65 ± 2.47	6.48 ± 2.66
Clinical stage			
Remission	33	11.38 ± 3.16	9.78 ± 3.51
Active	76	19.27 ± 4.51[ab]	14.49 ± 3.51[abf]
Severity			
Mild	40	13.37 ± 2.89[a]	11.28 ± 4.57[a]
Moderate	24	19.18 ± 4.66[ac]	16.72 ± 4.16[acf]
Severe	12	28.15 ± 5.37[ac]	20.77 ± 5.63[acf]
Clinical type			
Early onset	37	16.28 ± 4.17[a]	11.79 ± 5.82[af]
Chronic relapsing	33	17.73 ± 6.55[ae]	12.66 ± 5.11[aef]
Chronic persistent	24	16.96 ± 5.18[ae]	11.84 ± 6.17[aef]
Acute fulminant	15	21.59 ± 7.62[ad]	16.49 ± 3.62[adf]

[a]$P < 0.05$ *vs* control; [b]$P < 0.05$ *vs* remission; [c]$P < 0.05$ *vs* mild; [d]$P < 0.05$ *vs* early onset; [e]$P > 0.05$ *vs* early onset; [f]$P < 0.05$ *vs* P-gp-positive.

Relationship between serum IL-8 levels and P-gp

Serum IL-8 levels were increased significantly in active mild, moderate, and severe disease, as well as in early onset, chronic persistent, and acute fulminant disease compared with the controls ($P < 0.05$). Serum IL-8 increased significantly in active *vs* remission, mod-

erate and severe *vs* mild, as well as in acute fulminant *vs* early onset disease ($P < 0.05$). In addition, IL-8 increased significantly in active, severe, early onset, chronic relapsing, chronic persistent, and acute fulminant disease in P-gp-positive patients compared with P-gp-negative patients ($P < 0.05$) (Table 7, Figure 5).

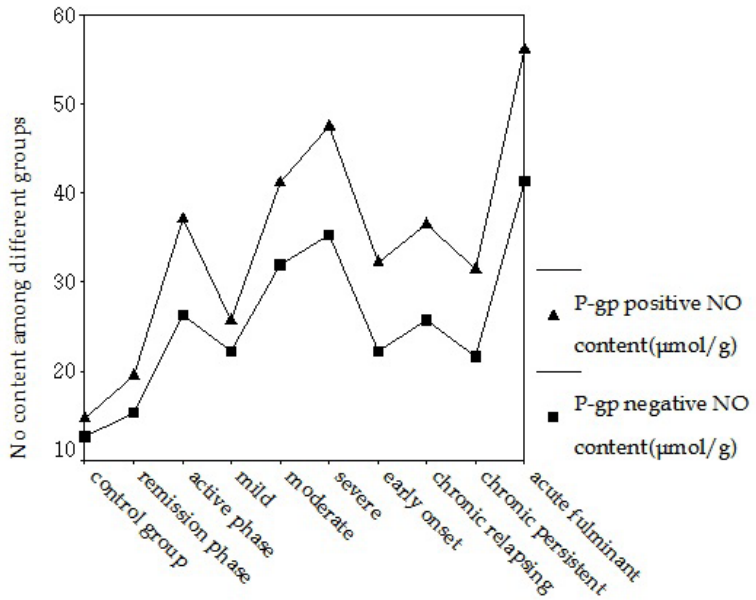

Figure 3. Comparison of NO content in colonic mucosa.

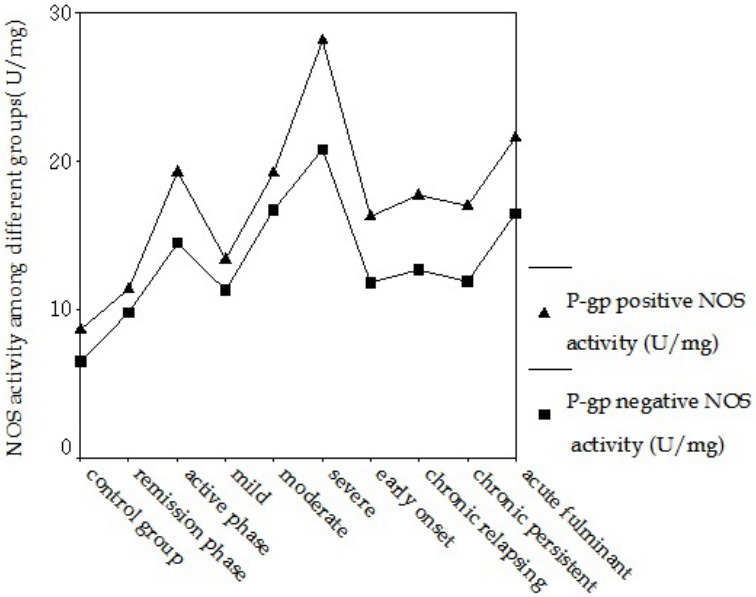

Figure 4. Comparison of NOS activity in colonic mucosa.

Table 7. Comparison of IL-8 levels in the different groups (means ± SD) (pg/mL).

Group	N	P-gp-positive	P-gp-negative	P
Control	35	134.63 ± 63.17	116.85 ± 43.71	
Clinical stage				
Remission	33	286.58 ± 57.82	237.67 ± 55.64	
Active	76	636.18 ± 49.31[ab]	499.14 ± 47.69[abf]	<0.05
Severity				
Mild	40	437.29 ± 67.58[a]	396.38 ± 66.28[a]	
Moderate	24	764.11 ± 64.13[ac]	757.16 ± 5.19[ac]	
Severe	12	978.57 ± 34.24[ac]	747.53 ± 9.82[acf]	<0.05
Clinical type				
Early onset	37	469.82 ± 68.53[a]	309.77 ± 58.34[af]	<0.05
Chronic relapsing	33	502.34 ± 40.89[ac]	311.22 ± 55.38[aef]	<0.05
Chronic persistent	24	491.42 ± 65.28[ac]	336.57 ± 42.81[aef]	<0.05
Acute fulminant	15	984.19 ± 68.74[ad]	751.54 ± 80.12[adf]	<0.05

[a]P < 0.05 *vs* control; [b]P < 0.05 *vs* remission; [c]P < 0.05 *vs* mild; [d]P < 0.05 *vs* early onset; [e]P > 0.05 *vs* early onset; [f]P < 0.05 *vs* P-gp-positive.

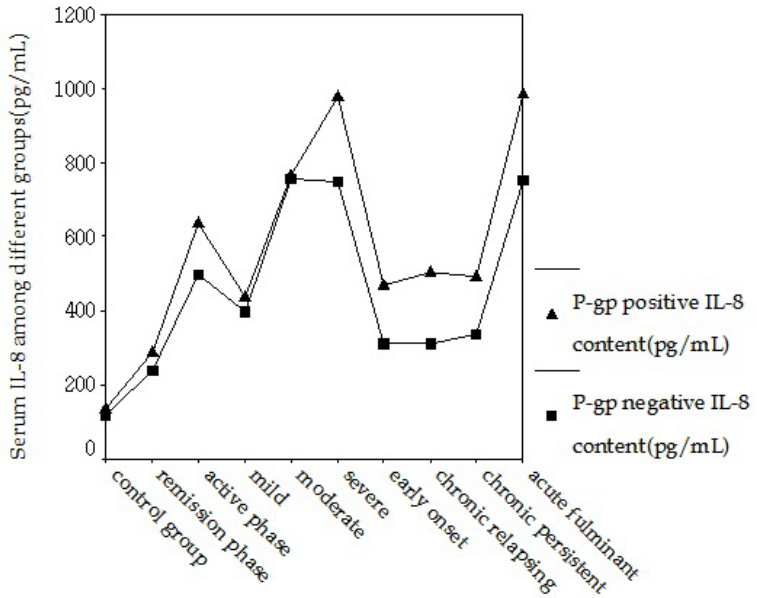

Figure 5. Comparison of serum IL-8 levels in different groups (pg/mL).

Relationship between peripheral blood CD4$^+$ and CD25$^+$ T cell subsets and P-gp

Peripheral blood CD4$^+$ and CD25$^+$ T cell subset percentages decreased significantly in active, mild, moderate, and severe disease, as well as in early onset, chronic persistent, and acute fulminant disease compared with in controls ($P < 0.05$). Peripheral blood CD4$^+$ and CD25$^+$ T cell subset percentages decreased significantly in active *vs* remission, moderate and severe *vs* mild, as well as in acute fulminant *vs* early onset disease ($P < 0.05$). Peripheral blood CD4$^+$ and CD25$^+$ T cell subset percentages decreased significantly in active, severe, early onset, chronic relapsing, chronic persistent, and acute fulminant disease in P-gp-positive patients compared with P-gp-negative patients ($P < 0.05$) (Table 8, Figure 6).

Table 8. Comparison of peripheral blood $CD4^+$ and $CD25^+$ T cell subset percentage (means ± SD).

Group	N	P-gp-positive	P-gp-negative	P
Control	35	0.84 ± 0.27	0.96 ± 0.19	
Clinical stage				
Remission	33	0.75 ± 0.12	0.87 ± 0.14	
Active	76	0.09 ± 0.27[ab]	0.44 ± 0.43[abf]	<0.05
Severity				
Mild	40	0.29 ± 0.38[a]	0.34 ± 0.28[a]	
Moderate	24	0.18 ± 0.31[ac]	0.20 ± 0.35[ac]	
Severe	12	0.05 ± 0.26[ac]	0.19 ± 0.82[acf]	<0.05
Clinical type				
Early onset	37	0.15 ± 0.51[a]	0.36 ± 0.34[af]	<0.05
Chronic relapsing	33	0.11 ± 0.34[ae]	0.32 ± 0.31[aef]	<0.05
Chronic persistent	24	0.17 ± 0.23[ae]	0.34 ± 0.57[aef]	<0.05
Acute fulminant	15	0.03 ± 0.25[ad]	0.17 ± 0.32[adf]	<0.05

[a]$P < 0.05$ *vs* control; [b]$P < 0.05$ *vs* remission; [c]$P < 0.05$ *vs* mild; [d]$P < 0.05$ *vs* early onset; [e]$P > 0.05$ *vs* early onset; [f]$P < 0.05$ *vs* P-gp-positive.

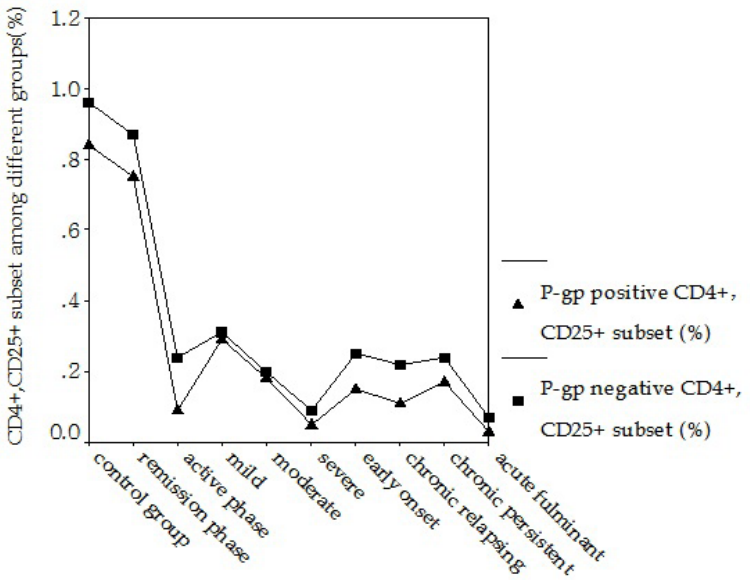

Figure 6. Comparison of peripheral blood $CD4^+$ and $CD25^+$ cell subset (%).

DISCUSSION

In this study, we found that the expression of P-gp in patients with UC before and after 6, 12, 18, and 24 months of treatment was lower than that in the control group. There was no difference in P-gp expression before treatment and 6 months after treatment; however, the rates of P-gp-positive patients after 12, 18, and 24 months of treatment were significantly different from those before treatment. These findings indicate that regardless of the type of UC, expression of the *MDR1* product, P-gp, is lower in UC patients than in normal healthy individuals. These findings also demonstrate that P-gp expression is not related to the duration of disease; as disease duration increased, P-gp became lower. It is unclear why P-gp expression was significantly lower in patients with acute fulminant UC.

Multidrug resistance is the most important cellular defense mechanism of tumor cells to avoid attack by chemotherapeutic agents (Gutmann et al., 2008), and multidrug resistance was initially identified in the study of drug resistance in tumor cells. However, P-gp is present not only in tumor cells, but also in normal cells (Fedier et al., 2007), and the results of this study showed that the multidrug resistance gene was present in normal controls, indicating intrinsic multidrug resistance.

Expression of the drug resistance gene was significantly lower in patients with UC before treatment and during early treatment than in controls. As disease duration increased, the drug resistance gene expression rate decreased; lowered P-gp expression can weaken the intestinal protective effect and increase disease activity.

P-gp expression rates in remission or active UC, regardless of disease severity, were significantly reduced compared with normal controls, and no differences were observed between remission and active disease or between patients with different disease severity. Therefore, irrespective of whether UC was in remission or active, P-gp expression was lower than that in normal healthy individuals. Similar results were demonstrated for the positive expression of P-gp in UC; however, this was not related to disease severity or active or remission status. The positive expression rates in all lesions were significantly different compared with normal controls. However, no differences were observed among lesions in different locations, i.e., straight sigmoid colon lesions, left colon lesions, extensive colon lesions, and rectal lesions. No significant difference in the P-gp expression rate was observed in UC patients without *vs* with extra-intestinal lesions.

These results suggest that expression of the *MDR1* product, P-gp, in UC is related to disease occurrence. Patients with UC may have inherent changes in drug resistance gene expression; the disease may occur with decreased P-gp expression and the extent of disease and extra-intestinal manifestations are irrelevant.

Studies examining autoimmune diseases and organ transplantation found that P-gp is the most important factor affecting the efficacy of corticosteroids, cyclosporin A, FK506, cyclophosphamide, azathioprine, and other immunosuppressive agents (Verbon et al., 2002). The high expression of P-gp in the peripheral blood lymphocytes of patients with systemic lupus erythematosus resulted indecreased lymphocyte glucocorticoid levels, steroid-resistance, and reduced efficacy (Hibi et al., 2003). A study by Wasilewska et al. (2007) also showed that steroid-resistance or -dependence in children with nephrotic syndrome was related to P-gp expression in peripheral lymphocytes.

The relationship between P-gp expression and the immune status of patients with UC in this study showed the following results: compared with normal controls, NO content and NOS activity in the colonic mucosa were significantly increased in active, active mild, moderate, and severe disease, as well as early onset, chronic relapsing, chronic persistent, and acute fulminant disease. NO content and NOS activity increased significantly in active *vs* remission, moderate or severe *vs* mild, and acute fulminant *vs* early onset disease, respectively. In addition, NO content and NOS activity were significantly increased in P-gp-positive patients compared to P-pg-negative patients with active, moderate, severe, early onset, chronic relapsing, chronic persistent, and acute fulminant UC. These results indicate that NO content and NOS activity in the colonic mucosa was significantly increased in UC independently of disease severity. In patients with high P-gp expression, increases in NO content and NOS activity were even more significant.

Increases in NO content and NOS activity were more significant in UC patients with high P-pg expression; these values increased with disease duration and severity. This may have resulted from recognition of the drug as a foreign body; therefore, P-gp plays a very important role in drug absorption and distribution in the body.

A previous study found that various drugs are substrates of P-gp and that P-gp substrates in the cytoplasm accumulates to high concentrations in the phospholipid bilayer and are then transferred to the substrate-binding site. ATP hydrolysis occurs at ATP-binding sites as an energy source, and the drug is then discharged into the extracellular region. As a result, P-gp reduces the oral bioavailability of substrates and MDR tumor cell toxicity, limiting the effects of the drugs (Hartz et al., 2004). Alternatively, this may also result from both internal drug resistance changes in UC and the development of inflammation (Salas et al., 2002).

Recent studies showed that endogenous NO is involved in the gastrointestinal mucosal barrier and the inflammatory process. NO is closely related with colonic disease and an NOS-inducer exists in UC. Increased iNOS and NO are characteristic of UC, and when subjected to certain cytokines, pathogenic microorganisms, and other stimuli for a few hours, a large amount of iNOS is generated, which leads to increased NO synthesis (Seril et al., 2007).

As a result of drug resistance, UC becomes refractory to treatment, leading to the spread of inflammation and disease progression. This, in turn, significantly increases NO content and NOS activity.

NO produced by catalytic arginine and structural NOS is thought to protect the intestinal mucosa, while NO produced by iNOS is toxic and increases inflammation. Decreased production of NO results in smooth muscle contraction, which is likely the most important factor causing bowel movement disorders and mucosal damage, indicating that, under certain circumstances, iNOS has a protective effect. Therefore, a small amount of NO is physiologically protective, while a large amount of NO increases inflammation and causes damage (Guihot et al., 2000).

IL-8 is primarily produced by mononuclear macrophages and endothelial cells when stimulated by other cytokines. Its main biological role is the chemotaxis of eosinophils, neutrophils, basophils, and T cells. It also promotes neutrophil lysosomal enzyme activity and phagocytosis, as well as induces neutrophil degranulation. IL-8 is a powerful leukocyte chemotactic factor and activator and an effective inflammatory chemokine. Subcutaneous injection of IL-8 into animals results in a large local accumulation of neutrophils and histopathological changes of acute inflammation (Sangfelt et al., 2002). The inflammatory response induced by IL-1, tumor necrosis factor (TNF)-α, and IL-6 is considered to be mainly mediated by chemokines, and IL-8 is one of the most important chemokines. This study demonstrated that IL-8 was significantly increased in active, mild, moderate, severe, early onset, chronic relapsing, chronic persistent, and acute fulminant UC compared to controls ($P < 0.05$). Serum IL-8 levels increased in active *vs* remission, moderate and severe *vs* mild, and acute fulminant *vs* early onset disease ($P < 0.05$). In addition, serum IL-8 levels significantly increased in active, severe, and acute fulminant UC in P-gp-positive patients compared to in P-gp-negative patients ($P < 0.05$). Higher P-gp expression in UC was correlated with higher IL-8 levels, which was more obvious as the duration and severity of UC increased. This may be either the result of drug treatment or the result of an interaction between the internal resistance of UC and the development of inflammation. Fan et al. (2009) investigated the relationship between inflammatory bowel disease activity and serum IL-8 and TNF-α levels and showed that serum IL-8 and TNF-α increased in UC patients compared to those in remission and normal controls. Increased IL-8 and TNF-α contribute to mucosal inflammatory damage. Drug resistance in

UC increases inflammation and significantly increases NO, NOS, and IL-8. P-gp is an important physiological transporter protein, and is mainly involved in normal tissue detoxification, removal of toxic substances *in vivo*, hormone secretion, transport of certain substances, and the protection of normal tissue cells against damage caused by exogenous toxins. Drugs are considered by the body to be foreign substances; thus, P-gp also plays a very important role in drug absorption and distribution. P-gp can discharge drugs from the cell; therefore, P-gp can reduce the oral bioavailability of substrate drugs (Ufer et al., 2009).

Various studies have suggested that abnormal immune and inflammatory responses in the intestinal mucosa play an important role in the pathogenesis of UC, and T cells are the primary immune cells involved in these responses. UC is an autoimmune disease involving immune response, genetic, environmental, and other factors. T cells are key immune cells in normal intestinal mucosal immune responses (Sheikh et al., 2008). T cells exert their effect mainly through T cell antigen receptor-mediated antigen recognition, activation, cytotoxicity, cytokine production, and secretion to protect the intestinal mucosa, remove damaged epithelial cells, resist the invasion by foreign antigens, and balance the intestinal environment so that the intestine can function normally. If there is an imbalance in the intestinal mucosal immunity and inflammation, this will inevitably lead to intestinal mucosal damage and colitis.

The results of this study showed that the peripheral $CD4^+$ and $CD25^+$ subset percentages significantly decreased in active, mild, moderate, and severe disease, early onset, chronic relapsing, chronic persistent, and acute fulminant UC compared to normal controls. The peripheral $CD4^+$ and $CD25^+$ subset percentage decreased significantly in active *vs* remission, moderate and severe *vs* mild, and acute fulminant *vs* early onset disease ($P < 0.05$). The peripheral $CD4^+$ and $CD25^+$ subset percentage also decreased significantly in active, severe, and acute fulminant disease in P-gp-positive patients compared to P-gp-negative patients.

In normal mucosal tissues, effector T cells and regulatory T cells are in a state of dynamic equilibrium to maintain intestinal mucosa homeostasis and immune system stability. If there are too many effector T cells or enhanced immunogenicity, the effect of effector T cells will surpass that of regulatory T cells; if there is a decrease in the number of regulatory T cells or functional abnormalities, both can upset the balance between the two, leading to mucosal injury and inflammatory bowel disease (Sumida et al., 2008). Among the peripheral T-cell subsets, $CD4^+$ T cells have been identified as the main effector cells leading to intestinal inflammation and are the main cell type found in mucosal tissue infiltration in all colitis models. In some studies, inflammation was improved after removal of $CD4^+$ T cells from the body, and specific expression of the forkhead/winged helix transcription factor in $CD4^+$ and $CD25^+$regulatory T cells can effectively prevent UC (Sitohy et al., 2008). Regulatory T cells include Trl, which secretes high levels of IL-10, and Th3, which mainly secretes TGF-β. In addition, forkhead/winged helix transcription factoris specifically expressed in $CD4^+$ and $CD25^+$ regulatory T-cells. $CD4^+$ and $CD25^+$ regulatory T-cells play an important role in the development of Th3 for Trl. Regulatory T cells differ from helper T cells; Thl and Th2 are T cells with immune regulation function and play an important regulatory role in a variety of autoimmune diseases.

Regulatory T cells were first reported by Sakaguchi et al. (2004). Direct cell contact and the release of TGF-β, IL-10, and other cytokines can suppress autoreactive T cells and reduce Th1 functions, which is important in immune tolerance and maintaining the balance of immune function.

This study also confirmed that immune injury plays a very important role in the patho-

genesis of UC. This results because the entire immune system, including NO content and NOS activity, significantly increases inflammation. The inflammatory effect of IL-8 and $CD4^+$ and $CD25^+$ regulatory T cells on immune and inflammatory responses in the intestinal mucosa was also confirmed. Multidrug resistance gene overexpression in UC may result from either intrinsic lesion development or drug-induced secondary resistance. UC becomes refractory because of drug resistance with progression of inflammatory lesions. As a result, NO content and NOS activity increase, and the inflammatory effects of IL-8, as well as the effect of $CD4^+$ and $CD25^+$ regulatory T cells in the immune and inflammatory responses in the intestinal mucosa are significantly enhanced. This multi-drug resistance gene overexpression is related to the duration and inflammatory activity of UC.

CONCLUSION

Expression of the multidrug resistance gene product, P-gp, is closely related to the duration of UC; the longer the duration of UC, the lower the P-gp expression. Expression of the multidrug resistance gene and its product, P-gp, is found in normal colon tissue and is closely related to UC. Decreased expression of the multidrug resistance gene and P-gp may cause UC. In patients expressing the multidrug resistance gene and P-gp, immune injury plays a more important role in the pathogenesis of UC.

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