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• *CLINICAL RESEARCH* •

Significance of glucocorticoid receptor expression in colonic mucosal cells of patients with ulcerative colitis

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Abstract

AIM: Glucocorticoid (GC) resistant ulcerative colitis (UC) remains a serious disease and is difficult to manage. Although the molecular basis of GC insensitivity is still unknown, GC receptors (GRα and GRβ) may play an important role in it. This study was aimed to investigate the relationship between the expression of GRα and GRβ in colonic mucosal cells of patients with UC, the efficacy of GC therapy and the intensity of inflammation.

METHODS: Twenty-five cases of UC were classified into: GC sensitive (n = 16) and GC resistant (n = 9) cases. Patients consisted of mild (n = 6), moderate (n = 8) and severe (n = 11) cases. GRα and GRβ expression in colonic mucosal specimens were investigated by immunohistochemistry, and compared between GC resistant and sensitive groups, and also among various degrees of inflammation.

RESULTS: All cases were positive for GRα and GRβ expression. Both positive association between GRα expression and the response of UC to GC and strong negative association between GRβ expression and the response of UC to GC were identified. There was no significant association between GRα/GRβ expression and the degree of inflammation of UC.

CONCLUSION: These findings suggest that both GRα and GRβ may play an important role in the action of GC, and that GRβ functions as a dominant negative inhibitor of GRα. Expression of GRα and GRβ in colonic mucosal cells of patients with UC may serve as predictors of glucocorticoid response, but can not function as markers of inflammatory intensity.

Key words: Glucocorticoid; Ulcerative colitis

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INTRODUCTION

Glucocorticoid resistant ulcerative colitis (GRUC) is a challenging clinical problem associated with life-threatening disease progression. Glucocorticoid (GC) treatment can be effective on ulcerative colitis (UC)[1], however, in relapsed cases, the conditions are frequently refractory even when a high dosage of GC is administered[2,3]. It is well known that a long-term use of GC often causes serious side effects[4]. It would be useful if the responsiveness of patients to GC could be evaluated before the administration. The molecular basis of GC insensitivity is still unknown. Delineation of the molecular basis for GC resistance is critical for the development of new treatment approaches for this group of refractory patients, and may provide new insights into the pathogenesis of chronic inflammation.

The fact that GC hormones and their receptors act in concert has led some investigators to study the role of the GC receptor (GR) in patients with chronic inflammatory diseases such as UC[5], asthma[6], systemic lupus erythmatosus[7], and nephritic syndrome[8].

The GC receptor is essential for GC action on various effector cells. In humans, there are two highly homologous isoforms of GR: GRα and GRβ. Both GRα and GRβ are products of alternative splicing of the primary transcript of GR messenger RNA (mRNA). GRα is a ligand-activated transcription factor that modulates the expression of glucocorticoid-responsive genes by glucocorticoid response elements (GREs), whereas GRβ does not bind to glucocorticoids and is transcriptionally inactive[9]. Bamberger *et al*[10], suggested that GRβ might be an endogenous inhibitor of GC action and an important dominant negative regulator determining GC sensitivity in the target tissues. Honda *et al*[5], reported that the expression of GRβ mRNA in peripheral blood mononuclear cells (PBMCs) might serve as a novel predictor of GC response in ulcerative colitis. If the above speculation about the dominant negative role of GRβ as correct, we could predict the GC resistance in diseased conditions, such as ulcerative colitis by examining GRα, GRβ and GRα/GRβ.

Previous discussion of topical therapy for ulcerative

colitis has implied a topical action and GC, which have poor systemic bioavailability, are still therapeutically effective. Some trial results suggest that plasma concentrations are unimportant[11]. According to Fiocehi[12], epithelial, mesenchymal and endothelial cells actively participate in intestinal inflammation, and play an important role in the pathogenesis of gut inflammation. Compared with circulating T cells, mucosal T cells are more susceptible to fas-mediated apoptosis, a physiological process of cell death that, if altered, could contribute to inflammatory bowel disease (IBD)[13]. Mucosal immunity disorder is critical to the development of UC. Topical steroids may improve inflammation mainly via mucosal cells.

In contrast to the situation in PBMCs of patients with ulcerative colitis, to the author's knowledge, there are no reports on GRα and GRβ expression in colonic mucosal cells. Hence, this study was undertaken to investigate GRα and GRβ expressions in colonic mucosal cells and their correlations with the response to GC, and the degree of inflammation.

MATERIALS AND METHODS

Patient selection

Twenty-five cases of ulcerative colitis were collected from the Department of Gastroenterology of West China Hospital, Chengdu, China. The age of the patients ranged from 16 to 55 years (mean±SD, 38.6±12.5 years). Based on the scoring systems for clinical symptoms and endoscopic findings, according to Rachmilewitz[14], patients were classified as GC-sensitive (n = 16) or GC-resistant (n = 9) after GC administration. Disease activity was divided into mild, moderate and severe ones based on colitis activity index (CAI)[15].

Immunohistochemistry

Paraffin wax embedded sections (5-μm thick) were mounted on APES coated slides. After dewaxed, sections were immersed in methanol containing 0.3% hydrogen peroxide for 25 min to block endogenous peroxidase activity. Slides were pretreated with an antigen retrieval method by heating in an autoclave with 1% citrates. After being rinsed in PBS, the slides were preincubated with normal goat serum (diluted 1:20 in PBS) for 15 min. The anti-GC receptor polyclonal antibodies were used at the dilutions mentioned in Table 1, and incubated for three hours at 37 ℃. Subsequently, the slides were incubated with biotinylated rabbit anti-human antibodies (Dako, glostrup, Denmark) diluted 1:300 in PBS/ BSA for 30 min, followed by incubation with streptavidin biotinylated horseradish peroxidase complex (1: 300 dilution) (Dako, glostrup, Denmark) for 45 min. 3' 3-diaminobenzidine was used as chromogen and haematoxylin as the counterstain.

For appropriate negative controls, the primary antibodies were replaced by PBS. The cytospin of peripheral blood mononuclear cells of patients with GC resistant ulcerative colitis was used as a positive control.

Image analysis

After immunostaining, the slides were examined under the light microscope. Nuclear or cytoplasmic staining was taken as positive. GRα and GRβ expressions on intestinal mucosal cells of ulcerative colitis were scored as follows: (1) Intensity of staining. Slides were assessed for the average degree of staining under moderate power (×200) and scored as follows: 1, weak staining; 2, moderate staining; and 3, strong staining; (2) The percentage of cells with positive staining was counted under high power (×400) and the following scores were allocated: score 1 <33.3%, score 2 = 33.3-66.7%, score 3 >66.7%.

The scores from (1) and (2) were added together to give a final score ranging from 0 to 6, designated as negative or positive as follows: -, score of 0; +, scores of 1-2, ++, scores of 3-4; +++ scores of 5-6.

Table 1 Details of primary polyclonal antibodies used against GRα and GRβ

Antibody	Antibodies against	Source	Clone	Dilution	Positive control
P-20	GRα	Santa Cruz	sc-1002	1:100	PBMC
Ab-1	GRβ	Oncogene	pc171	1:400	PBMC

Table 2 GRα and GRβ expression in ulcerative colitis with different response

	Expression of GRα[a]					Expression of GRβ[a]				
	-	+	++	+++	total (n)	-	+	++	+++	total (n)
Resistant	0	3	5	1	9	0	0	1	8	9
Sensitive	0	0	7	9	16	0	4	5	7	16

There is a significant association between GRα/GRβ expression and the response of ulcerative colitis to GCs ([a]P<0.05).

Statistical analysis

To evaluate the significance of the investigation, χ^2 test and Fisher's exact test were applied as appropriate. All P values were based on two-tailed statistical analysis, and P values tess than 0.05 were considered statistically significant. All analyses were performed using the SPSS statistical software (SPSS Inc, Chicago, Illinois, USA)

RESULTS

Table 2 summarizes the results of the two markers tested in the two categories of ulcerative colitis. Nuclear or cytoplasmic staining of the intestinal mucosa cells was counted as positive. Staining in PBMCs was taken as a positive control. In our study, GR existed mainly in intestinal mucosal interstitial inflammatory cells, but GRβ appeared mainly in epithelial cells. The intensity of staining varied between individual intestinal mucosal cells. All the 25 ulcerative cases studied, were positive for both GRα and GRβ (Figure 1). A positive association between GRα expression and the response of ulcerative colitis to GCs was identified. Different GRα expressions (+, ++ and +++) were observed in 3, 5 and 1 case of GC resistant patients, respectively; but in GC sensitive patients different GRβ expressions (+, ++ and +++) were observed in 0, 7 and 9 cases, respectively (P<0.05). A strong negative association

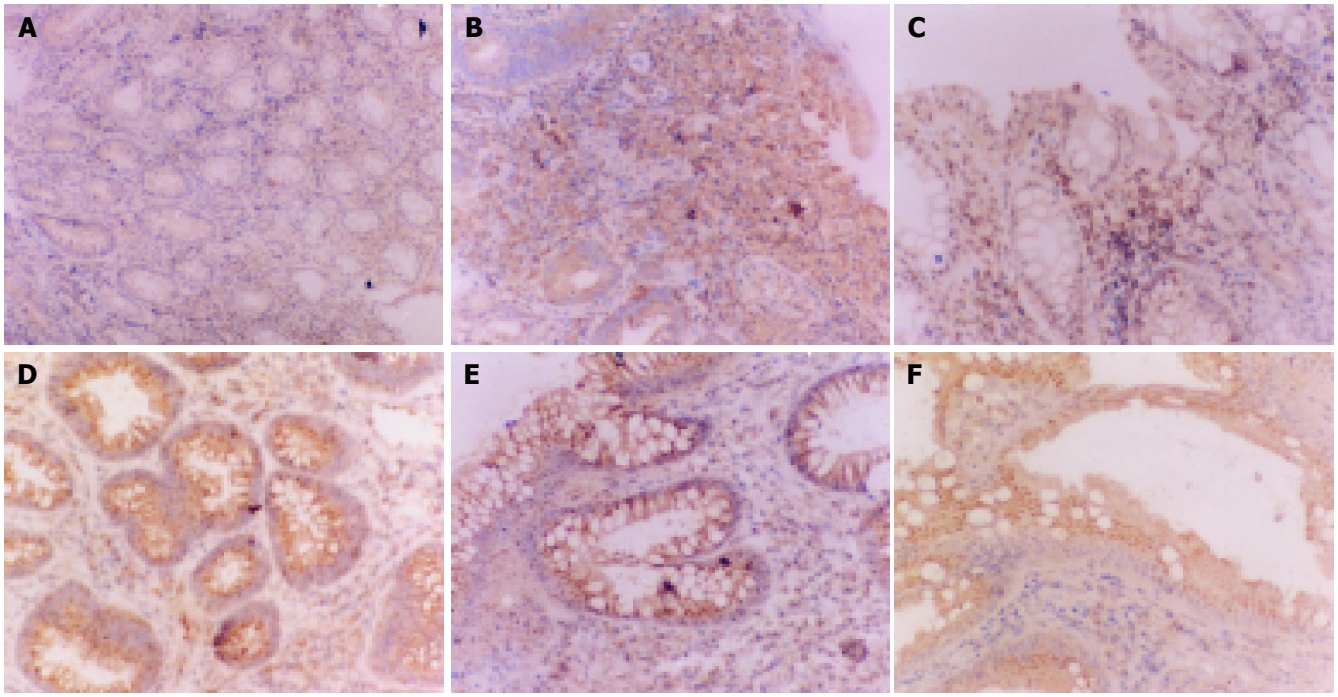

Figure 1 GRα expression in colonic mucosal cells of UC. A: GRα expression (+) in GC resistant UC, severe degree of inflammation (×100); B: GRα expression (+++) in GC sensitive UC, moderate degree of inflammation (×100); C: GRα expression (++) in normal control (×200); D: GRβ expression (+++) in GC resistant UC, severe degree of inflammation (×200); E: GRβ expression (+) in GC sensitive UC, moderate degree of inflammation (×200); F: GRβ expression (++) in normal control (×200).

between GRβ expression and the response of ulcerative colitis to GCs was observed as well. The GRβ expressions (+, ++ and +++) were observed in 0, 1 and 8 cases of GC resistant patients, respectively; but in GC sensitive patients the GRα expressions (+, ++ and +++) were observed in 4, 5 and 7 cases, respectively (P<0.05). No significant association between GRα/GRβ expression and the degree of inflammation of ulcerative colitis was found (Table 3).

DISCUSSION

GRα and GRβ are thought to be the result of alternative splicing of a single gene. Sequence analysis indicates that the α and β isoforms are 777 and 742 amino acids respectively. They are identical up to amino acid 727, after which they diverge. GR (P-20) is an affinity-purified rabbit polyclonal antibody raised against a peptide mapping at the carboxyl terminus of GC receptor α of human origin. GRβ (Ab-1) is a rabbit polyclonal antibody generated by immunizing rabbits with a synthetic peptide corresponding to amino acids 728-742(NVMWLKPESTSHTLI) within the C-terminal domain of human GC receptor β. Ab-1 has previously been shown to be specific for GR-β with no cross-reactivity against GRα[6,16].

Table 3 GRα and GRβ expression in ulcerative colitis with different severity

	Expression level of GRα[a]					Expression level of GRβ[a]				
	-	+	++	+++	total (*n*)	-	+	++	+++	total (*n*)
Mild	0	1	2	5	8	0	1	3	4	8
Moderate	0	1	7	3	11	0	2	2	7	11
Severe	0	1	3	2	6	0	1	1	4	6

There is a significant association between GRα/GRβ expression and the degree of inflammation of ulcerative colitis ([a]P<0.05).

In our study, the patients were categorized into GC sensitive and GC resistant cases according to Rachmilewitz[14]. Immunohistochemistry analyses of the slides were carried out by means of P-20 and Ab-1, respectively. We found that all patients with ulcerative colitis had GRα, GRβ proteins in their intestinal mucosal cells, regardless of the therapeutic effects of GC. In addition, there was an association between immunoreactivity of GRα/GRβ and the GC therapeutic effects. However, Sousa *et al*[17], investigated the expression of α and β- GC receptor isoforms in tuberculin-driven cutaneous cell-mediated inflammatory lesions in people with asthma, and found that the mean number of cells expressing GRα immunoreactivity in the lesions evoked in GC-sensitive and -resistant patients with asthma was statistically equivalent (93/1985 and 160/1 306). The number of cells expressing GC receptor β was significantly elevated in the patients who were GC resistant (26/1985 and 122/1306). Honda *et al*[5], reported that GRα mRNA was detectable in PBMCs of all patients with ulcerative colitis, whereas GRβ mRNA was detectable in a few UC patients. Furthermore, GRβ expression showed a significant negative association to GC sensitivity.

Our findings agree with those of Honda *et al*[5], Schottelius *et al*[18], and Hamid *et al*[19], in that immunoreactivity for GRα was moderate in GC resistant patients, but strong in GC sensitive patients. Our findings also agree with those of Loke *et al*[20], and Liu *et al*[21], in that the immunoreactivity for GRβ was higher in GC-resistant patients than in GC sensitive patients. According to Honda *et al*[5], although every

patient expressed GRα regardless of GC sensitiveness or resistance, all GC sensitive patients did not express GRβ. However, in our study, all patients regardless of being sensitive or resistant to GC expressed GRβ. The most important difference between GC sensitive patients and GC resistant patients lay in the quantities of GRβ.

Glucocorticoid receptors were reported to be localized in the cytoplasm of all somatic cells[18]. The concentration of GR varies between different tissues and even within a given tissue; receptor levels may fluctuate with changes in the cell cycle[22], during aging[23], and in response to hormone exposure[24]. In addition to technical differences, the difference in the number and nature of cases studied may explain the disagreement between our study and those of others. Further studies of a larger series of cases of GRUC are needed to confirm a weak association of GRα/GRβ with therapeutic effects of GCs.

In addition, no association was found between GRα/GRβ expression and the severity of ulcerative colitis. The fact that GRβ-positive and GC-resistant patients responded to other immunodulatory therapies indicates that GRβ expression is not correlated with disease severity of UC or a direct indication of proctocolectomy[5]. Our findings also agree with Honda *et al*[5], in that GRα/GRβ expression could not act as markers of activity of ulcerative colitis.

In conclusion, it seems that all ulcerative colitis cases are positive for GRα or GRβ. It indicates the need for further investigation of GRα or GRβ status, in addition to their conventional protein expression. This could yield potentially useful information for establishing new therapeutic strategies and evaluating the prognostic outcome in patients with ulcerative colitis, and the therapeutic effects of GCs via intestine.

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