ORIGINAL RESEARCH

Glucocorticoid receptor expression and glucocorticoid therapeutic effect in nasal polyps

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Abstract

Purpose: To investigate the expression and quantity of glucocorticoid receptor- α and - β in polyp tissues taken from the patients treated were subsequently treated with topical glucocorticoid (GC).

Methods: Eighty patients with nasal polyps were initially enrolled in the study. All polyp specimens were obtained prior to treatment. Patients then received daily topical GC spray treatment for one month. Polyp specimens were tested for glucocorticoid receptor (GR) GR- α and GR- β mRNA expression using fluorescent quantitative-reverse transcription-polymerase chain reaction (FQ-RT-PCR). Thirty healthy nasal mucosa tissue samples were tested at the same time.

Results: Forty patients finished the study and were divided into two groups: GC-sensitive ($n=26$) and GC-insensitive ($n=14$), according to treatment results. GR- β mRNA expression in the nasal polyp tissues of the GC-insensitive group ($5.72 \pm 0.58 \times 10^2$ copies/ μg) was higher than that in the GC-sensitive group ($4.82 \pm 0.28 \times 10^2$ copies/ μg , $P < 0.05$) and in the normal nasal mucosa group ($4.44 \pm 0.35 \times 10^2$ copies/ μg , $P < 0.01$). There was also a difference in the relative expression of GR- α and GR- β between the GC-sensitive group (GR- α /GR- β = 829.42 ± 67.36) and the GC-insensitive group (535.7 ± 89) ($P < 0.01$).

Conclusion: GR- β mRNA was highly expressed in patients with nasal polyps. Down-regulation of GR- α mRNA suggests the existence of glucocorticoid insensitivity. Expression of GR- β may play an important role in the evaluation of the glucocorticoid therapeutic effect in patients with nasal polyps.

Glucocorticoid (GC) resistance or insensitivity exists in a number of diseases, including asthma and ulcerative colitis. In our experience, we have encountered patients with nasal polyps who do not benefit from GC treatment. Integration of the inflammatory response within the upper and lower respiratory tracts has been proposed^{1,2}, and with the identification of and research on steroid-resistant asthma, studies on GC resistance in nasal polyps are of great clinical importance.

In recent years, studies have shown that the glucocorticoid receptor- β (GR- β) exerts an antagonistic effect on glucocorticoid receptor- α (GR- α)^{3,4,5}; therefore, GR- β may play an important role in GC insensitivity. In our study, we investigated the expression of GR- α and GR- β , and the relationship between GR expression and glucocorticoid therapeutic effects in patients with nasal polyps.

Materials and Methods

Patient selection

This study was approved by Ethics Committee of Sun Yat-Sen University and we obtained informed consent from the patients.

Eighty patients (56 males and 24 females) with nasal polyps, identified by anterior rhinoscopy or nasal endoscopy in the out-patient department of our hospital, were enrolled in the study. Patients had no history of allergic rhinitis, asthma, aspirin sensitivity, previous surgery for chronic sinusitis or pregnancy. The average age was 42 years old. Nasal polyp specimens were obtained via nasal endoscopy. Before tissue sampling, all patients avoided GC systemic administration (for at least two months) or GC local administration (for at least one month).

The normal nasal mucosa was obtained from the inferior turbinate mucosa from 30 patients with deflection of nasal septum (without history of allergic rhinitis) who underwent surgical treatment in our hospital. The controls included 22 males and 8 females, with an average age of 31 years old. None of them had a history of nasal or sinus disease, allergic rhinitis or upper respiratory tract infection, and none had GC treatment for at least 4 weeks prior to surgery.

All specimens were stored immediately at -80°C for future use. Fluticasone propionate (200 µg/d) was administrated once a day. One month after the GC local treatment, the therapeutic efficiency of GC was evaluated as described by Jankowski *et al.*⁶

Of the 80 patients originally identified, 40 (27 males and 13 females, mean age 39.2 years) were screened for GC efficacy at the end of the study. Twenty-six cases had a score >3 - the most sensitive group for GC therapy, and 14 cases had a score <1 - the most insensitive group for GC therapy.

Amplification primers and fluorescent probes

GR-α primers:

sense:

5'-TGAAAATGGGTGGTGCTTCTA-3',

antisense:

5'-GACAAGAATACTGGAG
ATTTGAGTCAA-3';

fluorescent probe:

5'-FAM-CCTGATGGCACTTAGCTATC
AGAAGACCACAA-TAMRA-3'.

GR-β primers:

sense:

5'-TGGCCACCCCAAAGGA-3',

antisense:

5'-GAGCTCATCCCATGCTAATTATC C-3';

fluorescent probe:

5'-FAM-AACTAACATGATTTGTG
TCTATGAAGTGC-TAMRA-3'.

The amplified fragments of GR-α and GR-β were 85 bp and 72 bp, respectively. Primers and probes were synthesized by Da'an Gene Company of Sun Yat-sen University.

RNA extraction and identification

Total RNA was extracted from the tissue with TRIzol (100 mg tissue plus 1.5 ml TRIzol) (Invitrogen, USA). The absorbance (A value) at 260 nm was measured with UV spectrophotometer (UV mini1240, Shimadzu, Japan). The formula for the total RNA concentration was shown as follows: concentration (g/L) = A₂₆₀ × dilution time × 40/1000.

Reverse transcription-polymerase chain reaction (RT-PCR)

RNA (2 µL) was taken as the template. Reverse transcription reaction was performed in PE9600 PCR machine (RT-PCR kit was purchased from QIAGEN, Germany). Reaction conditions were 37 ° C for 1 h, followed by 3 min at 95 ° C.

Fluorescent quantitative PCR

The reaction system was shown as follows: 5 times diluted PCR buffer (ABI, U.S.A) 10 μ L; forward primer (25 μ mol/L) 1 μ L; reverse primer (25 μ mol/L) 1 μ L; dNTPs (10 mmol/L) (Sigma, U.S.A.) 1 μ L; fluorescent probe (20 μ mol/L) (Shanghai Sangon Biological Engineering Technology And Service Co., Ltd., China) 1 μ L; Taq enzyme (ABI, U.S.A.) 2 μ L; cDNA 5 μ L; ddH₂O (double-distilled water or sterile ultrapure water) 29 μ L. Components of PCR buffer: 10 mmol/L Tris-HCl (pH 8.0); 50 mmol/L KCl; 2 mmol/L MgCl₂. Reaction conditions were as follows: 93 °C for 2 min, 93 °C for 1 min, 55 °C for 1 min, with 40 cycles. The system was reacted in PE 7000 automatic fluorescence quantitative PCR machine (Perkin Elmer, U.S.A). When the reaction was finished, the fluorescence signal was automatically analyzed and initial copy number, Ct, of GR- α / β was calculated. The fluorescent quantitative PCR results (copy/ μ L cDNA) was then converted copy/ μ g cDNA based on the concentration of RNA, and the amount of GR- α / β RNA (copy/ μ g cDNA) of samples was obtained.

Fluorescence gradient amplification curves curve and standard curve

With 10-fold dilutions of positive standards, from 10⁴ to 10⁷, four diluted standard templates were put into different reaction tubes respectively. After amplification using PE7000 fluorescent PCR system, standard templates and positive samples showed the classical "S"-type curve (Figures 1a and 2a), whereas negative controls showed flat lines. The concentrations and the Ct' values of the standard templates produced the regression curve and regression equation automatically. The horizontal coordinate of the standard curve represented the logarithm of concentration of the standard templates and the vertical coordinate represented Ct. Based on the regression equation, the system calculated the concentration of the unknown samples under the value of its Ct (Figures 1b and 2b).

Statistical analyses

Statistical analyses were performed with SPSS13.0 software. After Levene's test of homogeneity of variance, the data of these three groups were analyzed with One-way ANOVA. SNK method was adopted for paired comparison. $P < 0.05$ was considered statistically significant.

Results

Expression of GR- α mRNA/ GR- β mRNA

Both GR- α and GR- β mRNA transcripts were expressed in nasal polyps tissues and normal nasal mucosa. GR- β mRNA expression in the nasal polyp tissues of the GC-insensitive group ($5.72 \pm 0.58 \times 10^2$ copies/ μ g) was higher than either the GC-sensitive group ($4.82 \pm 0.28 \times 10^2$ copies/ μ g) or the normal nasal mucosa group ($4.44 \pm 0.35 \times 10^2$ copies/ μ g), and there was significant difference between these two treated groups ($P < 0.05$). The results also showed significant difference of GR- α /GR- β ratio between the GC-sensitive group (829.42 ± 67.36) and the GC-insensitive group (535.7 ± 89) ($t = 11.74$, $P < 0.01$) (see Table 1). The correlation coefficients for standard curves of GR- α and GR- β were 0.997 and 0.996.

Discussion

Glucocorticoids act through the GR to either enhance or repress transcription of glucocorticoid responsive genes, depending on the promoter context and cellular background. The human GR primary transcript is alternatively spliced, resulting in GR- α and GR- β isoforms. Transactivation and transrepression are both mediated by GR- α . Within the last few years, a number of studies have focused on the structure and function of the GR- β isoform. GR- β differs from GR- α in its carboxy terminus, where the last 50 amino acids of GR- α are replaced by a non-homologous 15-amino acid sequence. GR- β neither binds glucocorticoids nor transactivates target genes.⁷ Transfection studies re-

FIGURE 1. Expression of GR- α mRNA. (A) Gradient amplification curves for GR- α fluorescence quantification, (B) Standard curve for GR- α fluorescence quantification

A

Fluorescence gradient amplification curves -GR- β

B

Standard curve of GR- β

FIGURE 2. Expression of GR- β mRNA. (A) Gradient amplification curves for GR- β fluorescence quantification, (B) Standard curve for GR- β fluorescence quantification

TABLE 1. Expression of GR- α /GR- β mRNA in control, glucocorticoid-sensitive and glucocorticoid-insensitive patients

Groups	Cases	GR- α mRNA $\times 10^4$ (copies/ μ g)	GR- β mRNA $\times 10^2$ (copies/ μ g)	GR- α /GR- β
Normal mucosa group	30	115.25 $\pm$ 5.04	4.44 $\pm$ 0.35	2614.28 $\pm$ 150.00
Sensitive group	26	40.12 $\pm$ 4.70	4.82 $\pm$ 0.28	829.42 $\pm$ 67.36
Insensitive group	14	30.43 $\pm$ 4.97	5.71 $\pm$ 0.56	535.70 $\pm$ 89.00
F value		2228.711	55.049	2386.399

GR- α mRNA expression:

Insensitive group vs. sensitive group: $t=6.12$, $P<0.05$;
 Insensitive group vs. normal mucosa group: $t=52.42$, $P<0.01$;
 Sensitive group vs. normal mucosa group: $t=57.40$, $P<0.01$.

GR- β mRNA expression:

Insensitive group vs. sensitive group: $t=-6.65$, $P<0.01$;
 Insensitive group vs. normal mucosa group: $t=-9.19$, $P<0.01$;
 Sensitive group vs. normal mucosa group: $t=-4.65$, $P<0.01$.
 There was difference in quantitative expression of GR- β mRNA ($P<0.05$).

Ratio of GR- α /GR- β :

Insensitive group vs. sensitive group: $t=11.74$, $P<0.01$;
 Insensitive group vs. normal mucosa group: $t=47.89$, $P<0.01$;
 Sensitive group vs. normal mucosa group: $t=55.95$, $P<0.01$.
 There was difference in the ratio of GR- α /GR- β ($P<0.05$).

vealed the ability of GR- β to act as a strong negative inhibitor of GR- α activity through a mechanism that involves the formation of transcriptionally impaired GR- α -GR- β heterodimers.^{7,8}

In our study, the GR- β mRNA expression was found to be higher for the GC-insensitive group in comparison with the GC-sensitive group ($P<0.05$). Leung *et al.*⁹ and Honda *et al.*¹⁰ examined the GR- β expression in peripheral blood mononuclear cells of patients with asthma and ulcerative colitis by both RT-PCR and immunohistochemistry, and found that the GR- β expression in peripheral blood mononuclear cells in the GC-uncontrolled group was significantly higher than both the GC-controlled group and the normal population. In addition, Goleva *et al.* found that GR- β expression was also increased in GC-resistant asthma.⁵ Thus, the results from these three previous studies are consistent with our data. Both GR- α mRNA quantitative expression, and the ratio of GR- α / β , were different between the GC-sensitive group and the GC-insensitive group, and between the insensitive group and the normal mucosa group. Both the average expression of GR- α and the ratio of GR- α / β in the insensitive group were lower than the sensi-

tive group and the control group. Other studies reported that a large number of inflammatory cells such as T lymphocytes and eosinophilic granulocytes exist in nasal polyp tissues, and that GR- β mRNA is highly expressed in these inflammatory cells.¹¹⁻¹³ Our study also showed that GR- β expression in all the nasal polyp epithelium (the sensitive group and the non-sensitive group) was higher than that in the normal mucosa ($P<0.05$).

In contrast, other investigators found no evidence for a specific dominant negative effect of GR- β on GR- α activity.¹⁴⁻¹⁶ These conflicting results may be explained by the different methodologies used. First, immunohistochemical studies do not accurately reflect the GR- α to GR- β ratio of the cell. In addition, absolute quantification of GR- α and GR- β proteins by Western blotting may not be technically accurate enough to determine the proportion of each receptor isoform.¹⁷ The conventional RT-PCR, which was most frequently used, is only a qualitative or semi-quantitative end-point detection method: the initial template amount can not be accurately calculated from the final product amount. In comparison, fluorescence quantitative PCR is a relatively accurate

method for quantitative analysis: there is a direct relationship between the intensity of the fluorescence signal and the amplification products at each time point, and the initial product can therefore be quantified directly. Because its sensitivity range is 0-10 copies/ml, fluorescence quantitative PCR can reflect the product amount of the target gene in tissue more scientifically than the conventional PCR. In Pujols's study, the patients with nasal polyps were divided into GC-treated and untreated groups.¹⁶ Even though there were steroid-insensitive individuals in the GC-treated group, because of the relatively small number, quantitative values of GR α and GR β expression from such patients were likely masked by GR α and GR β expression level in the majority of GC-sensitive patients. It may therefore be difficult to extrapolate from that data the actual quantitative levels of expression of the GR α and GR β in the steroid-insensitive patients. This may help explain why results appeared to differ in that study.

Based on the increased expression of GR- β has been reported in patients with glucocorticoid-insensitive asthma and other diseases.³⁻⁵ Therefore, according to the results of our study, the level of GR- β mRNA expression and the ratio of GR- α to GR- β may reflect the effect of GR- β on GR- α activity within the nasal cavity. If overexpression of GR- β mRNA accompanied lower ratio of GR- α to GR- β was found in nasal polyps, the result may show the condition of glucocorticoids insensitivity in the patient's nasal cavity, which might result in poor effectiveness of local GC treatment. Otherwise, if the nasal polyps have the expression of GR- β mRNA and the ratio of GR- α to GR- β in normal level, local GC treatment for the patient might be effective because of glucocorticoid-sensitive condition in nasal polyps. So, before the therapy of local GC treatment for the patients with nasal polyps, testing their level of GR- α/β expression in nasal cavities might be significant for evaluation of glucocorticoid therapeutic effect in patients with nasal polyps.

Currently, local GC treatment is still one of the effective methods for nasal polyps. Therefore, it is necessary to explore the mechanism of GC insensitivity for nasal polyps, which will supply a basis for new treatment strategy for the nasal polyps that is refractory to GC treatment.

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