Glucocorticosteroid therapy in asthma modulates the expression of histamine receptors mRNA in peripheral lymphocytes

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Abstract

Among a numerous pathophysiological influences, histamine modulates an immunological response through stimulation of an appropriate receptor. It acts proinflammatory through H1R and immunosuppressively through H2R. During wheezing in asthma, the concentration of histamine in blood is considerably increased. Glucocorticosteroids (GCS) are the most efficient drugs in the therapy of asthma due to their potent anti-inflammatory and immunosuppressive activity.

We investigated the expression of four histamine receptors mRNA in peripheral lymphocytes of 7 healthy subjects, 8 asymptomatic (untreated) and 14 GCS-treated asthmatics (12.0 ± 2.5 mg/daily). The study was performed on isolated lymphocytes using RT-PCR technique. Primers were labelled with fluorescent dyes. Data were analysed using ABI Prism sequencer and Gene Scan collection software, version 3.1. Intensity of fluorescence was expressed in relative fluorescence units.

It was found that the expression of H1 and H4 mRNA did not differ between the groups in contrast to H2R mRNA expression, which was significantly lower in untreated asthmatics than in control ($p < 0.01$). In treated asthmatics this expression was similar to those in healthy subjects and significantly higher than in untreated asthmatics ($p<0.001$). Expression of H3R mRNA was similar in healthy and untreated subjects whereas significantly higher in treated patients ($p<0.02$). An increase in expression of H2R mRNA may be an important element in the mechanism of action of GCS considering documented participation of histamine, through H2R, in suppression of immunological response.

Key words: *asthma, histamine receptors mRNA, glucocorticosteroids*

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Introduction

Bronchial asthma is a chronic inflammation of respiratory airways in which the key part have mastocytes, eosinophils and T lymphocytes. The most common form is genetically determined atopic asthma which pathogenetic mechanism includes reaction of allergen with IgE bound to the receptors on mastocytes and basophils. As a result of the reaction, numerous mediators are released, including histamine.

Histamine is the main mediator of ongoing allergic inflammation in asthma. An increased concentration of histamine was found in plasma of asthmatics following specific and non-specific provocation [1], during exacerbations of asthma [2] and also in bronchoalveolar lavage (BAL) in asymptomatic subjects with mild asthma [3].

Histamine has diverse pathophysiological influence depending on the type of stimulated receptor. The four human histamine receptors (HR) have been cloned [4-7] which enabled the application of RT-PCR technique in studies on their function. Biological role of histamine receptors is the best known so far for H1R and H2R. Histamine exerts its proinflammatory activity, through H1R [8] whereas it acts suppressively on many elements of immunological response through H2R [9, 10].

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Glucocorticosteroids (GCS) are recognized as the most efficient drugs in treatment of asthma due to their potent anti-inflammatory and immunosuppressive action. In a long-term therapy their inhalatory form is recommended whereas systemic application takes place during long-continued treatment of severe asthma or during exacerbations. However, having beneficial influence on the course of asthma, GCS exert numerous different, unwanted side effects [11, 12].

The aim of our study was the investigation of the influence of GCS therapy on the expression of four type HR in peripheral blood lymphocytes of asthmatic subjects.

Materials and methods

The study was performed on lymphocytes of 7 healthy subjects aged 26.1±5.2 years (3 females, 4 males), 8 asymptomatic asthmatics (3 females, 5 males aged 33.8±11.7) not receiving any medication for three months (except for β_2-agonist prn), mean duration of the disease 13.5±10.2 years and 14 asthmatic patients (aged 43.7±12.6 years, 8 females, 6 males) – mean duration of the disease 15.6±9.0 years remaining on sustained systemic steroid therapy, mean daily dose converted to prednisone 12.0±2.5 mg.

Lymphocytes were isolated from heparinized (10 IU/ml) blood by density gradient centrifugation [13]. Monocytes were removed using plate method [14]. Viability of cells assessed by trypan blue exclusion was >98%. Immediately after, the total RNA was isolated from 2.5 x 10^6 cells using trizol method (Trireagent™ LS, Sigma, St Louis, MO, USA) following the protocol of the manufacturer. Determinations of mRNA of four HR in particular subjects were done at the same time. Reverse transcriptase polymerase chain reaction was performed using Titan™ One Tube RT-PCR System (Behringer, Mannheim, Germany) following strictly the instruction of the manufacturer. Fluorescent dyes labelled primers were obtained from Bionovo, Bioresearch Equipment and Biochemicals, Legnica, Poland. For H1R: sense 5'-JOE (6-carboxy-4', 5'-dichloro-2',7'dimethoxyfluorescein) – GCC TAT GAA TAT GAA CAT CCT CTA CCTGCT-3', antisense 5'-GCT CGG GTC TTG GTA CGA TAC TTA-3', for H2R: 5'-6-FAM (6-carboxyfluorescein) - GGT CAT CTC CAT TAC CCT GTC CTT-3', antisense 5'-GAC CTT GAA GAT GCG GTA GTA GGT-3', for H3R: sense 5'-TAMRA-CTT CGC AGT TAC TGG TTG GTG TTC 3', antisense 5'-GAG GCT TAT GCA AGA GAC AGA AGG 3' and for H4R: sense 5'-ROX-AGA TTG TTA CTC TGA TGG TGG CCG -3', antisense 5'-CGA CTA AGA TGA CTG GGA TCA CGA-3'. Amplification of cDNA was performed in the thermocycler (Termoreaktor DTC-15, Hiperon, Sieradz, Poland) through 35 cycles of duplication reactions (30 sec. 94°C, 30 sec. 53°C, 30 sec. 68°C). For instrumental analysis 1.5 µl of the reaction product with 18 µl deionized formamide was analysed, after thermal denaturation, in liquid polyacrylamide using the sequencer ABI Prism 310 and Gene Scan version 3.1 software. Peak area automatically expressed as relative fluorescence units (RFU) was the measure of activity of particular gene.

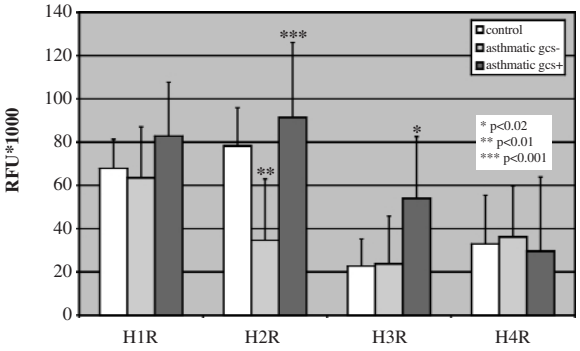

Fig. 1. Expression of mRNA of H1, H2, H3 and H4 histamine receptors in peripheral lymphocytes of healthy, untreated (GCS-) and glucocorticosteroids treated (GCS +) subjects

Statistical analysis

The data were expressed as mean ± standard deviation and comparison between the groups was performed using paired and unpaired Student t test, p values, 0.05 were considered significant.

Results

Comparison of the expression of mRNA of 4 types HR in peripheral lymphocytes of healthy subjects and asthmatics treated (GCS+) and untreated (GCS-) with GCS is shown on Fig. 1. The expression of H1R mRNA, although higher in treated patients, did not differ significantly between the groups.

Considerable, statistically significant differences in H2R mRNA expression were found. It was significantly lower in untreated subjects than in control group ($p<0.01$) and also significantly lower than in treated patients ($p<0.001$).

The expression of H1R and H2R mRNA in healthy subjects was similar with slight prevalence H2R mRNA. The ratio H2R/H1R mRNA was 1.15±0.2 in contrast to untreated asthmatics in which markedly lowered expression H2R mRNA in relation to H1R mRNA – 0.51±0.3 was found. The difference in H2R/H1R mRNA ratio between these groups was statistically significant – $p<0.001$. Close mutual expression of H2R and H1R mRNA in GCS-treated and healthy subjects was found. The ratio – 1.22±0.5 was significantly higher than in untreated asthmatics ($p<0.001$). The expression of H2R mRNA in treated asthmatics was significantly higher than in untreated ones ($p<0.001$) and not different from the expression in healthy subjects.

The expression of H3R mRNA was not different between healthy and untreated (GCS-) subjects whereas it was significantly higher in treated asthmatics (GCS+, $p<0.02$).

The expression of H4R mRNA was similar in all groups.

Discussion

It was found in our experiments that the expression of H2R mRNA in untreated asthmatic subjects was significantly lower ($p < 0.01$) than in healthy controls. The expression of H3R mRNA was higher in treated group ($p<0.02$). No differences were observed in the expression of H1R and H4R mRNA. Systemic therapy with GCS significantly changed the ratio of expression H2R/H1R mRNA which was lowest in untreated asthmatics and significantly different from those in the remaining groups ($p<0.001$). However, H2R mRNA expression in GCS-treated subjects was similar to the expression in healthy ones and significantly higher than in untreated asthmatics ($p<0.001$).

An increased expression of H2R mRNA in treated patients may be an important element in the mechanism of action of GCS considering documented participation of histamine in suppression of immunological response through H2R [9, 10, 15-18].

As it was shown, GCS changed the proportion of subpopulations CD4, CD8 in favour of CD8. It was also shown that such a change correlated with improvement in spirometric parameters, decreased eosinophilia and symptom-score [19, 20].

Recently we have reported the lowered expression of H2R mRNA in pollinosis, an atopic disease in which GCS also beneficially limit an inflammation [21]. Inage et al. [22], using E-rosette technique with HR agonists, observed markedly lower percentage of T lymphocytes with membrane expression of H2R in nephrotic syndrome patients, which significantly increased after steroid therapy ($p<0.01$).

It is known from clinical observations that coexistence of asthma and peptic ulcer is a rare phenomenon [23]. Histamine, through H2R, is the most potent stimulator of hydrochloric acid secretion by goblet cells. Gonzales et al. [24] reported the dysfunction of H2R in atopics. The authors studied the basic and maximal gastric output, finding significantly lower stimulated secretion of HCL in the latter ($p<0.01$).

Peptic ulcer disease is an undesirable side effect of a long-term, systemic GCS therapy [12]. Therefore the patients have often prescribed the drugs limiting gastric secretion. Most frequently these are the drugs from H2R antagonists group (i.e. ranitidine, famotidine). These drugs, blocking H2R present on all cells may simultaneously diminish therapeutic effect of GCS based, as observed here, on an increase in expression of H2R mRNA. For this reason we think that different drugs, i. e. proton pump inhibitors, would be more beneficial as peptic ulcer prophylaxis during GCS therapy.

Conclusions

1. The expression of H2R mRNA in peripheral lymphocytes of asthmatics was significantly lower than in healthy subjects.
2. Systemic, oral therapy with GCS increased the expression of H2R and H3R mRNA in asthmatics in comparison to untreated ones.
3. Observed influence of GCS therapy on the expression of HR mRNA may contribute to better understanding of particular stages in the mechanism of GCS action.

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