



The Effect of Short-Term and Chronic Glucocorticoid Therapy on Lymphocyte Glucocorticoid Receptor Number in Patients with Asthma

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Abstract. Glucocorticoid (GCs) hormones are widely used in the treatment of bronchial asthma. However, not all aspects of their pharmacological effects are well understood as yet. It is known that the effects of GCs are mediated through GC receptors (GCRs). We sought to evaluate the effect of short-term and chronic GC therapy on GCR number in peripheral blood lymphocytes, the relationship between GCR number and cortisol concentrations in asthma patients treated with GCs as well as the response to GC therapy in various pictures of this disease. Sixty-nine patients with bronchial asthma were investigated. Thirty-five of them had received steroid therapy: 18 patients for 1 to 15 years and 17 patients for 13 days after a prior 3-month discontinuation of steroid treatment. The control group consisted of 28 healthy, age-matched volunteers. GCR numbers were determined using tritiated dexamethasone as a ligand. The scatchard method was applied to calculate the maximal specific binding and the dissociation constant. The number of receptor sites per lymphocyte was calculated. Cortisol was measured by radioimmunoassay. Lymphocyte GCR numbers in patients with bronchial asthma who were not treated with steroids, did not differ from age-matched healthy persons (means 8115 ± 812 and 7905 ± 832). A significant decrease in receptor number was seen in patients receiving steroid therapy (mean 4331 ± 1041). There was also a significant difference in receptor number between the groups with short-course (mean 3741 ± 549) and chronic steroid therapy (mean 4885 ± 1095). The number of GCRs did not correlate with age, sex, clinical state or serum cortisol concentration in either group.

Key words: bronchial asthma; glucocorticoid therapy; glucocorticoid receptor.

Introduction

Since the discovery of glucocorticoids (GCs) over 50 years ago, efforts have been made to fully understand their mechanism of action and to elucidate the molecular mechanisms of their effects. It is well known that GCs alter the expression of target genes via interaction with GC receptors (GCRs).

Studies concerning the relation between response to

GC therapy and GCR content, most advanced in human lymphoid malignancies, have highlighted the role of GCR measurement for predicting the efficacy of GC administration. However, only a small number of investigations have focused on GCR number and function in asthma, in which GC therapy plays a vital role. Although inhaled GC therapy is the treatment of choice in asthma, patients with severe exacerbation of the disease may require systemic GC courses. Studies on GCR

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may enable the optimization of hormone dose and the appropriate identification of GC resistance status. The present study was undertaken to evaluate the effect of short-term and chronic GC therapy on GCR number in peripheral blood lymphocytes, the relation between GCR number and cortisol concentrations in asthma patients treated with GCs, as well as to relate their response to treatment and disease-dependent factors.

Materials and Methods

Subjects. Sixty-nine patients with stable, mild to severe asthma as estimated according to American Thoracic Society criteria were recruited for the study from the Department and Clinic of Internal Diseases, Allergology and Clinical Immunology of the University School of Medicine in Katowice, and the Out-patient Allergological Clinic in Zabrze. All patients demonstrated significant reversibility of bronchial constriction (at least 15% improvement after a standard dose of inhaled β_2 -agonist). Patients with symptoms of other diseases were excluded. The subjects were divided into two groups.

- Group 1 – 34 patients with mild asthma (22 men and 12 women) aged 18–52, mean 27.5 ± 7.9 years. The duration of the disease was 1–32 years, mean 11.2 ± 8.1 years. None of them had received GCs before or during the study. The asthma symptoms were controlled in 29 of them by sodium cromoglycate and β_2 -agonists. Five of them received no medication at least 2 months before the study. Twenty five showed no symptoms during the study, and 9 showed mild bronchial obturation ($70\% < FEV_1 < 80\%$, PEF variability 20–30%).
- Group 2 – 35 patients with moderate to severe asthma (13 men and 22 women) aged 26–52, mean 36.5 ± 8.7 years. The duration of the disease was 3–30 years, mean 14.3 ± 7.1 years. Prolonged or intermittent GC therapy had been administered to all patients of this group. Most of them had received sodium cromoglycate, β_2 -agonists, theophylline and mucolytics (intermittent or chronic therapy). This group was further subdivided according to the duration of steroid treatment:

– 17 patients who had been treated with short courses of GCs (up to 16 days per month, 1–8 times a year, mean 4.6 ± 2.3 years). Patients in this group had received GCs for at least 13 days (mean daily doses equivalent to 18.38 ± 12.21 mg prednisolone). The short course of GC treatment was started at the beginning of

asthma exacerbation. All patients demonstrated improvement in lung function following GC therapy (increase in the $FEV_1 > 15\%$). On the day of evaluation (after 13 days of GC therapy) 5 patients exhibited slight inspiratory wheezing without dyspnoea. Patients were excluded from this subgroup if they had taken any GCs within 3 months before the study started.

– 18 patients with severe asthma who had received GCs for 1 to 15 years, mean 4.2 ± 3.6 years. The following indications for prolonged therapy were applied: a marked improvement of asthma symptoms with low steroid doses and the inefficiency of other forms of treatment. GC were given in mean daily doses equivalent to 15.94 ± 8.9 mg prednisolone. All patients responded well to GC therapy. Only one person was unable to continue work and 4 required hospitalization for an exacerbation. On examination, 8 patients demonstrated respiratory murmurs. One patient had moderate disturbances in ventilation (borderline reversibility).

- The control group consisted of 28 healthy volunteers, 12 women and 16 men, aged 21–59 (mean 33.8 ± 8.5 years) with no history of smoking or medication (including vitamins and contraceptives).

In all subjects, blood samples for GCR and cortisol levels were drawn from the cubital vein at about 7 a.m., after a 30 min rest. In patients on GC therapy the sampling was performed 24 h after the last GC dose.

Reagents. The following reagents were used for GCR measurements: phosphate buffered saline (PBS) from Biomed (Poland); Ficoll (type 400) from Pharmacia (Sweden); Hypaque 60% from Polfa (Poland); May-Grunwald-Giemsa reagent; 1% trypan-blue in PBS from Biomed (Poland); (1,2,4-3H) dexamethasone ethanol solution from Amersham (England) – specific activity 41 Ci/mmol, total concentration 1 mCi in 1 ml ethanol, molar concentration $24.9 \mu\text{mol/l}$; unlabeled dexamethasone in substantia from Polfa (Poland), molecular mass 393.5; Soluen 350 from Packard (USA), 0.5 N quaternary ammonium hydroxide in toluene; and Aquasol “Universal LSC Cocktail” from Dupont. Cortisol concentrations were determined by the use of Steron-K- $^{125}\text{J-M}$ from Polynia (Russia).

Glucocorticoid receptor assays. Peripheral blood lymphocytes were isolated on Ficoll-Hypaque gradient according to BOYUM⁶. Cells in the interface were pooled and washed three times and resuspended in PBS at a density $2\text{--}11 \times 10^6$ cells/ml. Differential cell counts of Giemsa-stained smears showed 4–10% monocytes and polymorphonuclear leukocytes. Viability was determined by trypan-blue. The cells and their viability were counted after our procedure and loss was less than 10%.

A competitive binding assay with whole cells was used. Binding assays were performed using a modification of the method of LIPPMAN and BARR¹¹ and PETERSON et al.¹³ Tritiated dexamethasone was used as a ligand. A 100-fold excess of unlabeled steroid was used to determine the non-specific binding. Specific binding was calculated as the difference between total and non-specific binding. After incubation for 2 h at 37°C, 1 ml of cold PBS was rapidly added to each tube. Cell pellets were collected after centrifugation, then washed and extracted with 0.2 ml Soluen 350. Extracts were transferred to 5 ml Aquasol. Radioactivity was counted in a β -scintillation counter. The Scatchard method was applied to calculate the maximal specific binding and the dissociation constant (Fig. 1). The number of receptor sites per one lymphocyte was calculated. We present all data as means of duplicate samples.

Hormone analysis. Cortisol was measured by radioimmunoassay.

Statistical analysis. Statistical analysis was performed by one-way or two-way analysis of variance. Post-hoc comparisons were done by the HSD Tukey test. Linear regression analysis was also performed.

Results

GCR were quantified in peripheral blood lymphocytes by Scatchard analysis, which showed uniform binding sites (the Scatchard plot for each subject was linear). Maximal binding of tritiated dexamethasone, in the majority of cases, was observed at 50–75 nmol/l of added dexamethasone concentrations (Fig. 1).

The mean number of GCRs on a lymphocyte in the control group was 7905 ± 832 . In group 1 (asthmatics receiving no GC therapy) the mean GCR number was 8115 ± 812 . In group 2 (GC-treated asthmatics) the mean GCR number of 4331 ± 1040 was significantly

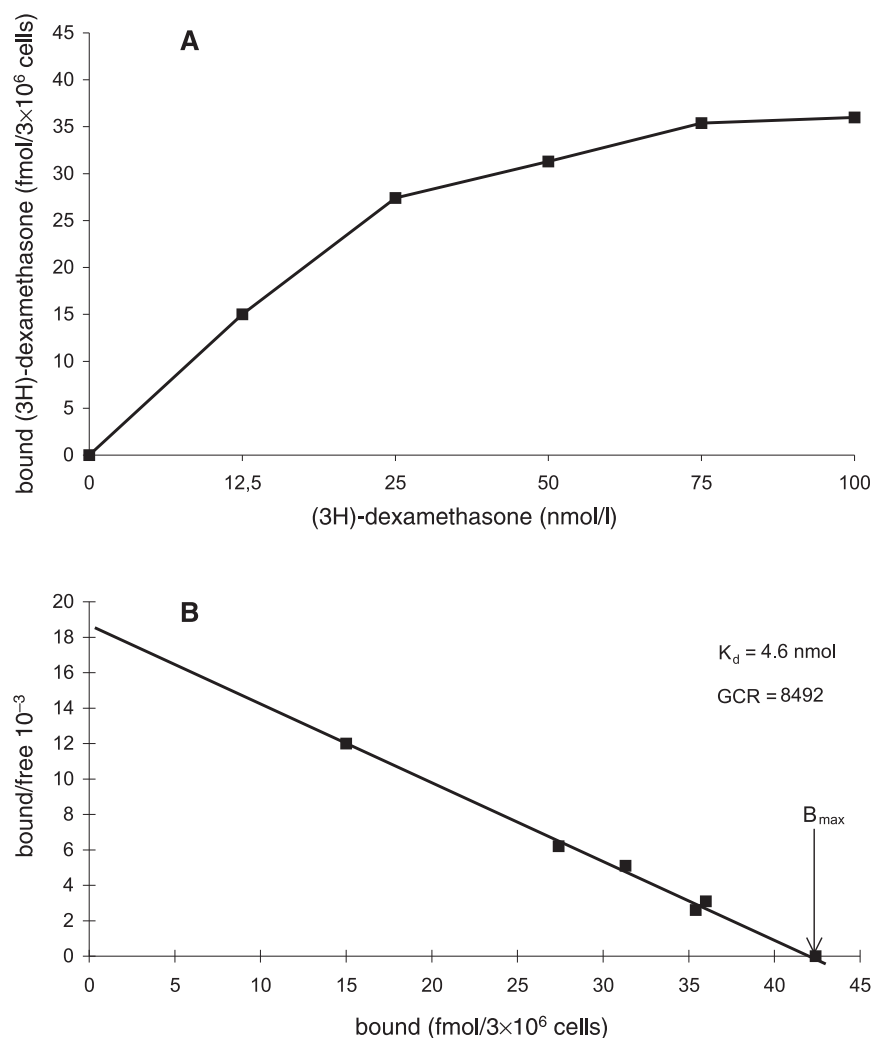


Fig. 1. Binding of (3H)-dexamethasone to human lymphocytes. **A** – shows specific-binding as a function of dexamethasone concentration added; **B** – shows maximal specific binding (B_{max}) and dissociation constant (K_d) calculated according to the Scatchard method

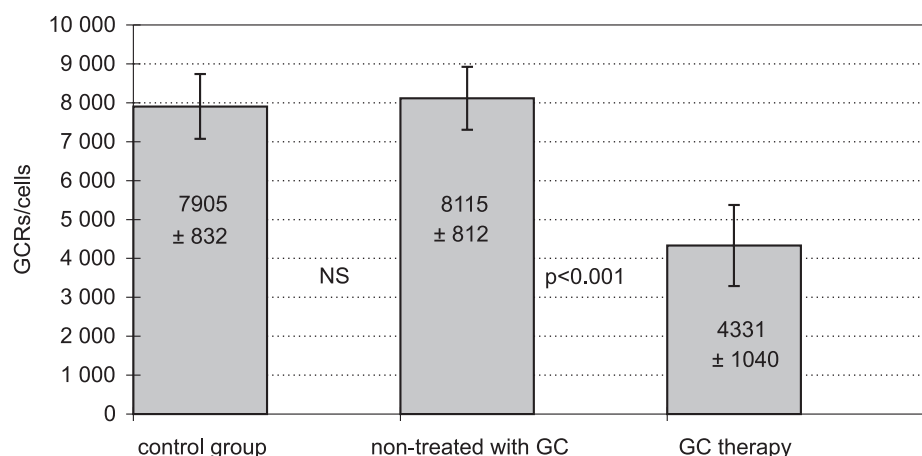


Fig. 2. Effect of GC therapy on lymphocyte GCR number in asthma. NS – not significant

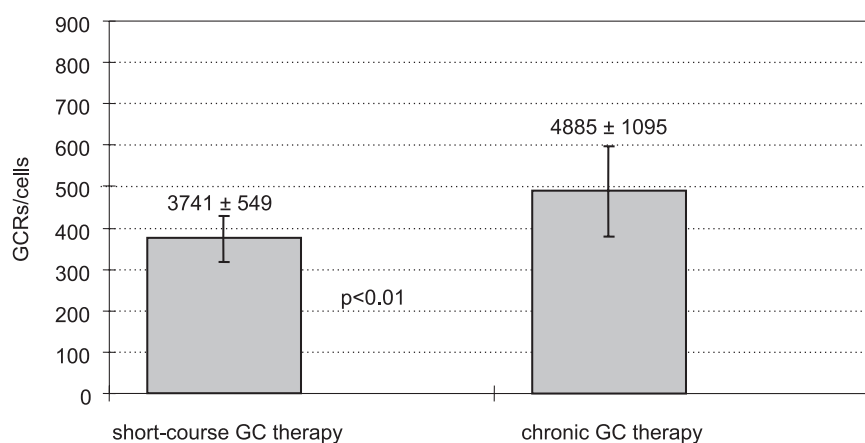


Fig. 3. GCR number in asthmatic patients in isolation for the duration of GC therapy

lower ($p<0.001$) than to that of group 1 (Fig. 2). There were differences in GCR number between the two subgroups in group 2. In patients on short-term GC therapy the mean number of GCRs after 13 days of treatment was 3741 ± 549 . Long-term GC therapy caused a smaller decrease, the mean number of GCRs being 4885 ± 1095 . The difference was found to be statistically significant, with $p<0.01$ (Fig. 3). The particular GCR values are given in Fig. 4. The above data suggest a relationship between GCR number and the duration of GC treatment. However, no linear correlation was observed in subjects on long-term steroid therapy.

The analysis of a relationship between GCR levels and GC doses calculated as prednisone equivalents showed no correlation either in group 2 as a whole or its subgroups.

Dissociation constants in controls and patients receiving no GC therapy were similar, the respective values being 5.08 ± 1.21 nmol and 4.43 ± 0.87 nmol. Administration of GCs caused a decrease in the binding affinity of dexamethasone. In patients on short-term GC

therapy there was a slight but significant ($p<0.05$) increase in the dissociation constant, up to 6.13 ± 1.98 nmol. Long-term GC treatment was found to reduce the binding affinity on average by about 50%. The dissociation constant in the latter subgroup was 11.59 ± 2.74 nmol. The difference was statistically significant, with $p<0.01$ (Fig. 5).

No correlations were observed between the number of GCRs and age, sex, or disease duration in any of the groups under study. Similarly, the number of GCRs was found to be unaffected by the use of bronchodilators in patients who did not receive GC therapy.

There was no statistically significant correlation between GCR number and clinical state in any of the patient groups, regardless of the therapy used. However, variance analysis suggests that some correlation is possible ($p=0.055$).

In group 1 (no GC therapy) the mean serum cortisol concentration was found to be 438 ± 109 nmol/l. In group 2 (GC-treated) the value was significantly lower ($p<0.001$), and there were differences in cortisol con-

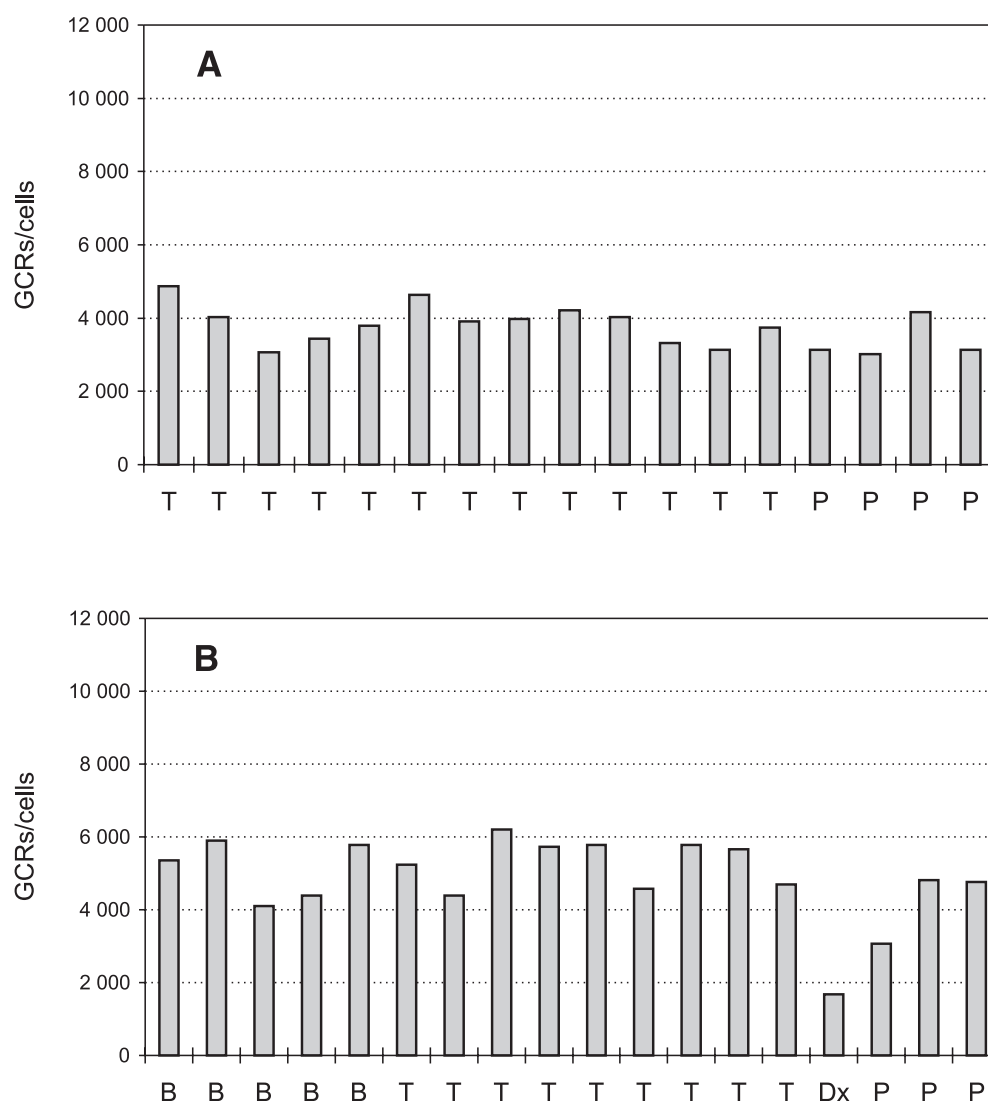


Fig. 4. Individual data of GCR number in patients with: **A** – short- and **B** – long-term GC therapy. B – betamethasone, T – triamcinolone, P – prednisolone, Dx – dexamethasone

centrations between patients on short-term and long-term GC therapy, the respective values being 201 ± 98 nmol/l and 132 ± 54 nmol/l ($p < 0.05$) (Fig. 6).

Regression analysis showed no correlation between cortisol concentration and the number of GCRs either in group 1 or group 2 as a whole. However, statistically significant correlations were observed in each subgroup of GC-treated patients. In those on short-term GC therapy the correlation coefficient was relatively low ($r = 0.49$, $p < 0.05$), while in those on long-term GC therapy it was higher ($r = 0.70$, $p < 0.01$) (Fig. 7).

Discussion

The number of GCRs in healthy controls, as determined by the method used, was 7905 ± 832 receptor sites

per lymphocyte, which was similar to that obtained by TSAI et al.¹⁶ The values described in the literature range 6000–11,000. The differences can be attributed to different methods and the labeled ligands used, incubation periods, percentage of other blood morphologic elements, and decreases in lymphocyte level and viability in the course of the study. GCR content also depends on the phase of cell cycle, e.g. a 2- to 3-fold increase in GCR number was observed for lymphocytes in the S phase compared with the G_0 and G_1 .⁸ Some authors determined only the maximal specific binding and reported the concentrations of ligand bound by cells, not receptor numbers.

No differences in lymphocyte GCR number were found between asthmatics who were not receiving GC therapy and healthy controls, which is in accordance with earlier investigations^{1, 16}. NAKAJIMA et al.¹² ob-

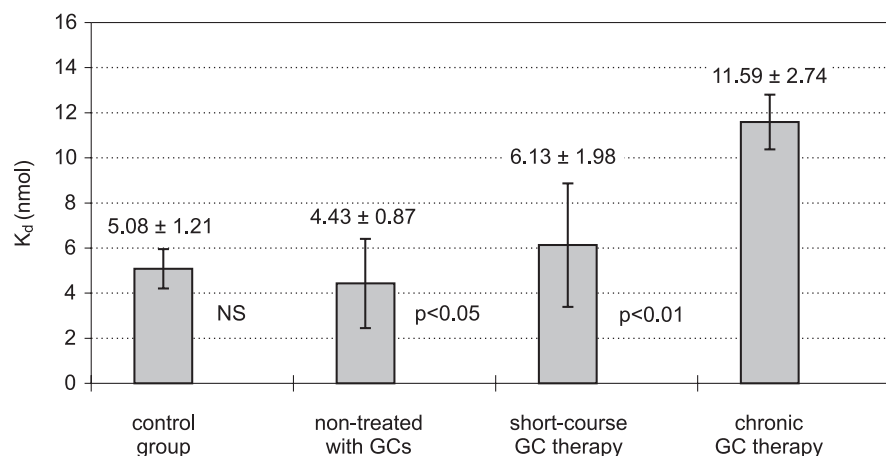


Fig. 5. GCR-binding affinities for dexamethasone in patients with asthma. NS – not significant

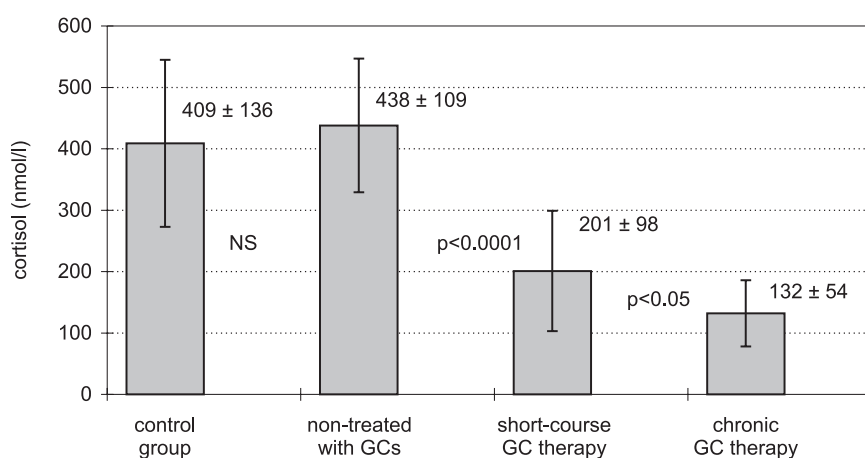


Fig. 6. Morning plasma cortisol concentrations in each group. NS – not significant

served a decrease in GCR content but the differences were not statistically significant. The above findings indicate that there are no GCR abnormalities in asthma. Similarly, there were no significant differences in GCR affinity for dexamethasone between the two groups, similar to the reports by other authors who also determined dissociation constants in patients with asthma without steroid-resistance and with nocturnal exacerbation^{10, 16}.

In patients who were not receiving GC therapy the effects of bronchodilators on GCR number were also examined and showed no correlation. Other studies indicate that high-dose β_2 -agonists may reduce the binding of GCRs to DNA⁴.

GC therapy was found to cause a significant decrease in lymphocyte GCR content. Our findings are in agreement with those reported by other authors³, but our study showed a smaller reduction in GCR number following prolonged GC administration compared with

a short course of therapy. There are no reports in the literature available comparing GCR number in patients on short- and long-term therapy. The down-regulation of the receptors is thought to be a mechanism for attenuating cellular responsiveness to GC excess⁵. The significantly higher number of GCRs in long-term treated patients compared with those in patients on short-term GC therapy suggests an “adaptation” to GCs. As no correlation was observed in the long-term GC therapy group between lymphocyte GCR content and treatment duration, it is possible that an initial marked decline in GCRs is followed by “adaptation” and no further changes occur. The higher GCR number in patients on long-term GC therapy may also be related to a lower GCR affinity for dexamethasone because of a nearly twice higher dissociation constant compared with that in the 2-week therapy group. According to CORRIGAN et al.⁷, however, *in vitro* differences in affinity do not reflect sensitivity to GCs, because the dissociation con-

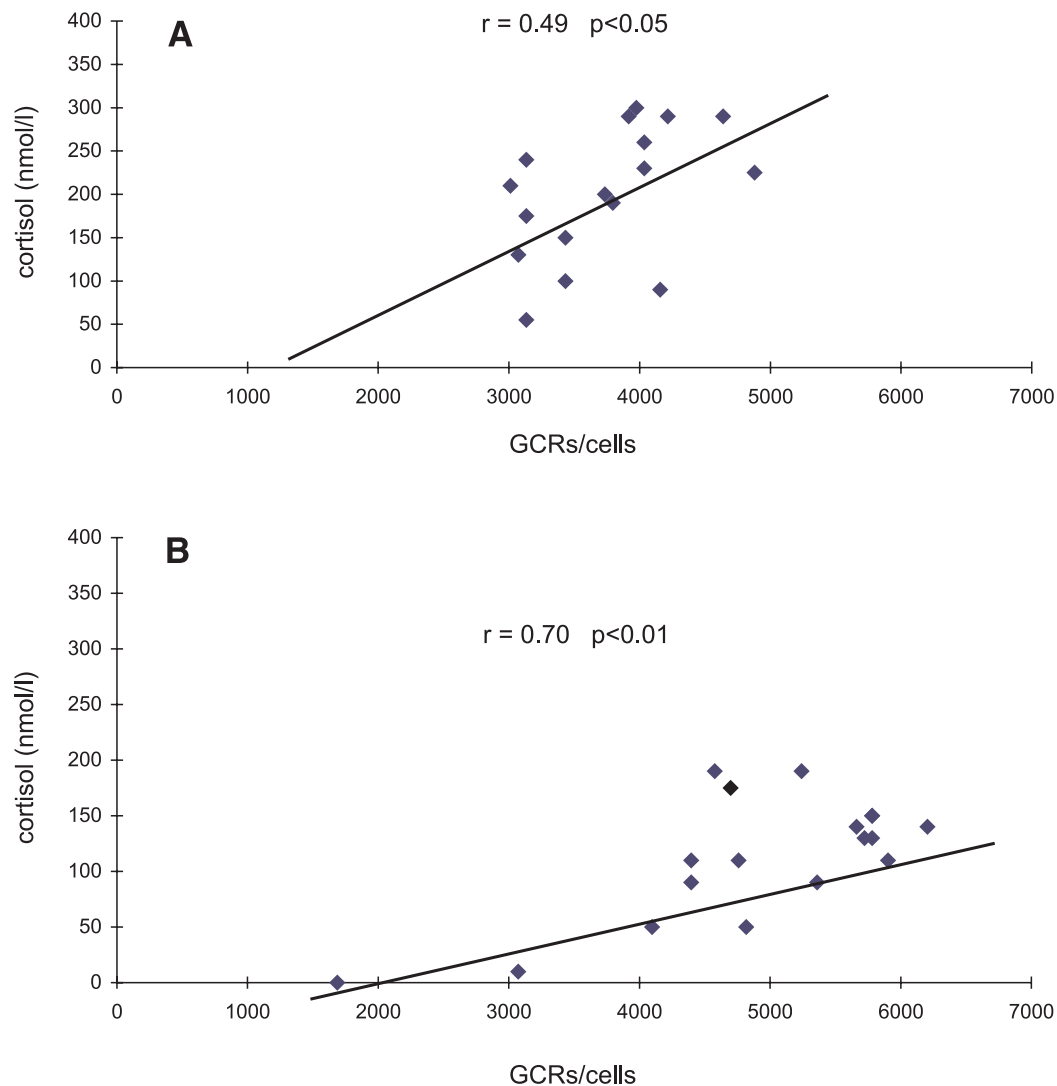


Fig. 7. Linear correlation between lymphocyte GCR number and cortisol concentration in patients with **A** – short- and **B** – long-term GC therapy

stant only expresses the free hormone concentration necessary to half-saturate the receptors. Authors who examined receptor affinity during GC administration generally found the affinity to decline. In our study, the 2-week GC therapy also caused a slight but significant increase in dissociation constant. None of the investigators determined K_d in the course of prolonged GC therapy (1–15 years) and compared the effects of short- and long-term therapy on GCR number in humans. There are, however, some animal data published. ALEXANDROVA², using an animal model, compared GCR response to a single dose of corticosterone and dexamethasone with the response to a 6-day administration of the steroids. A single dose of corticosterone reduced GCR concentration in the rat mammary gland cytosol to 60%, while the 6-day treatment reduced it only to 25%.

Conflicting data have been published on the effect of the different steroid preparations and the dose used

to calculate GCRs content. Some authors observed no correlation⁹, while others found the decrease in GCRs to be dependent on the dose¹² or the type of GC used². In the study of ALEXANDROVA², dexamethasone lowered GCR levels equally, regardless of the treatment duration. We did not analyse the influence of the GC type used because the groups receiving the same type of GC were too small to be compared. The correlation analysis between GCR number and the dose of GCs applied, calculated as the prednisone equivalent, did not show the number of receptors to be dose-dependent either in the whole GC-treated group or in its subgroups, which is in concordance with the findings of other authors⁹. NAKAJIMA et al.¹² determined the so-called borderline dose, 5 mg prednisolone daily, above which the maximal binding capacity is reduced. In our study the lowest dose used was equivalent to 7.5 mg prednisolone per day. Thus, a correlation between GCR levels and the

dose could probably be established in patients only receiving lower GC doses.

A correlation between GCR amount and the clinical index of disease severity on the day of the trial was determined in all patients with asthma and was borderline significant ($p=0.055$). Earlier studies concerning the estimation of the number of GCRs in asthma showed no impact of it on the clinical picture of the disease^{12, 16}. However, in another study¹⁰ patients with nocturnal asthma exhibited a significantly lower GC binding affinity at 4 a.m. Such correlation was reported in other diseases. SHARMA et al.¹⁵ observed a correlation between GCR number in the cells of bronchoalveolar lavage fluid and the presence of fever in patients with sarcoidosis. A decrease in lymphocyte GCRs was reported in patients with active rheumatoid arthritis and systemic lupus erythematosus, followed by an increase during remission¹⁴.

None of the groups studied showed correlations between the number of GCRs and sex or age, only TSAI et al.¹⁶ demonstrated sex-related GCR regulation in patients with asthma, the maximal number of GCRs being about 18% higher in male subjects than in female subjects.

Significant differences in cortisol concentrations between control and non-GCs-treated subjects and those receiving GCs confirm the suppressive effect of GCs on the secretory function of the adrenal cortex. It is well known that inhibition of the hypothalamic-pituitary-adrenal axis depends on the type of GC used, daily dose, duration and frequency of treatment courses, and route of administration. Significant differences in cortisol levels between patients on a 2-week and prolonged therapy clearly demonstrate the influence of these factors, particularly the effect of the treatment duration. There was no correlation between serum cortisol and GCR number in any of the groups studied, which is consistent with earlier reports¹⁴, including those regarding GCRs in asthma^{12, 16}. However, a correlation (between serum cortisol and GCR number) was observed when two subgroups of patients receiving either short-term or long-term GC therapy were analysed separately, thus indicating the influence of GCs on both the endogenous cortisol and the lymphocyte GCR levels. Lack of correlation in the whole GC therapy group, as shown by different regression plots for each subgroup, reflects different mechanisms of GCR adaptation, depending on the duration of GC treatment.

In conclusion, the number of GCRs in long-term GC therapy is reduced less than in short-term therapy, probably owing to the mechanisms of adaptation.

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