Down regulation of glucocorticoid receptors in early-diagnosed rheumatoid arthritis

A.A. van Everdingen[1,2], A.M. Huisman[1], M.J.G. Wenting[1], D.R. Siewertsz van Reesema[2], J.W.G. Jacobs[1], J.W.J. Bijlsma[1]

[1]Department of Rheumatology & Clinical Immunology, University Medical Center Utrecht; [2]Department of Rheumatology, Deventer and Zutphen Hospitals, The Netherlands.

Abstract

Objective

In patients with rheumatoid arthritis (RA) of longer duration, glucocorticoid receptor (GR) down-regulation has been reported without any change in cortisol levels. This phenomenon might play a role in the aetio-pathogenesis of RA. Therefore we studied GR expression, as well as the serum cortisol levels, in patients with recently diagnosed RA.

Methods

In 81 early diagnosed RA patients with disease duration < 1 year (52F/29M; mean (SD) age 63 (13) years) and in 39 age and sex matched controls (23F/16M; mean age 63 (15) years) blood samples were taken between 8-10h AM. GR expression (GR-number and GR-affinity), serum cortisol levels, ESR, CRP, painful and swollen joints were measured.

Results

A significantly lower GR-number was found in the female patients compared with female controls: 7.0 versus 9.8 fmol/million cells, respectively (difference: 2.8, 95% CI 1.1 - 4.6). Interestingly, also serum cortisol levels were significantly lower in the female patients compared with the female controls: 0.21 versus 0.41 mol/l, respectively (difference: 0.20, 95% CI 0.12 - 0.28). However, between the male patients and male controls no difference was found in GR expression nor in serum cortisol levels. Neither in female nor in male patients were correlations found between GR expression and parameters of disease activity nor was there a relation between GR expression and serum cortisol levels.

Conclusions

Changes in GR expression as well as serum cortisol were not a general phenomenon in early diagnosed RA patients, being present only in females and not related to disease activity. Therefore it seems unlikely that GR expression per se is causally involved in the pathogenesis of RA. We cannot preclude that it may be involved in the incidence, severity and course of RA, as this may be differentially regulated in males and females.

Key words

Rheumatoid arthritis, glucocorticoid, receptor.

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Please address correspondence to: A.M. Huisman, Rheumatology & Clinical Immunology (F02.127), University Medical Center Utrecht, PO Box 85000, 3508 GA Utrecht, The Netherlands.

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Introduction

Glucocorticoids are being successfully used in the treatment of early rheumatoid arthritis (RA) because of their potent anti-inflammatory action. Not all patients do respond however and if so, some patients respond to low doses, while others require larger doses for seemingly identical clinical situations. Glucocorticoids exert their effects through the glucocorticoid receptor (GR) located in the cytoplasm of target cells at low doses (1, 2), but at higher doses genomic (i.e. through the GR) as well as non-genomic modes of action play a role (3). It is known that the number of intracellular GRs per cell is closely related to the biological response upon glucocorticoid exposure to that cell (4). In this context the GR expression of peripheral mononuclear cells (PBMC) is considered to reflect *in vivo* biological effects of glucocorticoids in healthy persons and in a variety of disorders (5-9). GR in normal leukocytes do not show significant alterations within a day, in contrast to plasma cortisol levels (5). GR downregulation might hinder the effectiveness of the immune-hypothalamic-pituitary-adrenal axis in the control of inflammation and therefore might play a role in the aetio-pathogenesis of RA (6, 10).

Schlaghecke *et al*. showed a diminished GR-number per cell in mononuclear leukocytes of RA patients with active disease of longer duration (mean 6 years) compared with healthy controls (6,11). There were no differences in GR binding affinity or serum cortisol levels. No correlation was found between GR-number and age or sex, RA activity or serum cortisol. The decrease in GR-number in RA is compatible with impaired activity of the hypothalamic-pituitary-adrenal (HPA) axis (10, 12,13). The diminished receptor density in RA patients did not result in glucocorticoid resistance in the sense that proliferation and cytokine release (interleukine 1 and 6) of lymphocytes of RA patients and healthy controls were inhibited by glucocorticoids to the same extent (11). This study was done with relatively high glucocorticoid doses: adding glucocorticoids to PBMC, acting by GR, but probably also by the qualitatively quite different non-genomic (i.e., not GR-related) effects of glucocorticoids (3). In contrast to the diminished GRs in active RA patients, Sanden *et al.* showed in patients with rheumatic diseases an overall increase in the number of GRs compared with healthy controls. However, patients in this study had a variety of different rheumatic diseases with a large range in disease activity. The increased number of GRs decreased on glucocorticoid therapy in a dose-dependent way (14).

If GR downregulation plays a role in the aetio-pathogenesis of RA a diminished GR-number would not only be found in RA of longer duration (6), but especially in early-diagnosed RA. To investigate this hypothesis, we compared the GR expression as well as the serum-cortisol levels of early RA patients with those of age-and sex-matched healthy controls.

Patients and methods

Patients

Eighty-one consecutive outpatients with recently diagnosed (early) RA (disease duration <1 year) were included. All patients fulfilled the ACR criteria and were DMARD and glucocorticoid naive. 68% of the RA patients (55 out of 81) used non steroidal anti-inflammatory drugs (NSAIDs) and 74% (60 out of 81) were IgMRF positive. Age varied between 24 and 82 years with a mean (SD) age of 63 (13) years. The cohort consisted of 29 males [age 61 (12) years] and 52 females [age 64 (13)]. From these patients disease activity (ESR, CRP, number of tender and swollen joints) and serum cortisol levels were determined. From the last 50 included patients glucocorticoid receptor (GR) density and affinity were determined as well. This sub-population had a similar mean age [64 (12) years] and age distribution (varying from 29 to 82 years) as the entire group. Also the male/female ratio (19/31), and age distribution between both sexes [male 63 (13); female 65 (12)] were similar to the entire group. In addition, cortisol, GR-density and GR-affinity were determined from an age- and sex-matched group of healthy individuals.

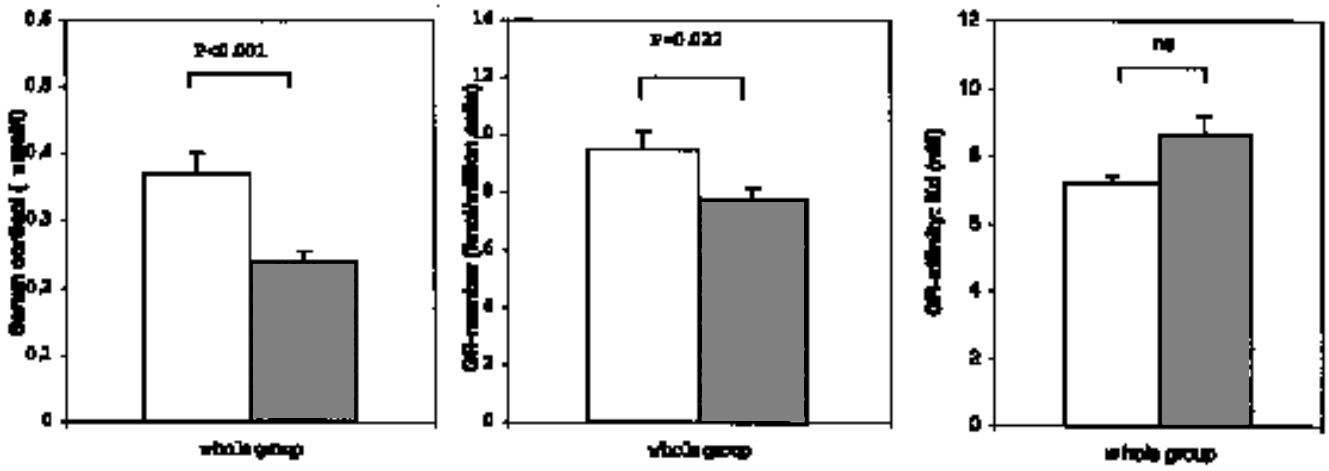

Fig. 1. (a) Serum cortisol levels (mol/l) of the healthy controls (open bars) compared with early RA patients (gray bars). Mean values (SEM) for the total control group 0.37 (0.03) and for the total early RA group 0.24 (0.015).
(b) GR-number:fmol/million cells. The open bars represent the healthy control group and the gray bars the early RA group. Mean values (SEM) for the total control group 9.48 (0.66) and for the total RA group 7.73 (0.41).
(c) GR-affinity (Kd) expressed in nM. The open bars represent the healthy control group and the gray bars the early RA group. Mean values (SEM) for the total control group 7.19 (0.48) and for the total RA group 8.64 (0.53).

This group included 39 individuals with a mean age of 63(15) years: 16 males [61 (12) years] and 23 females [62 (16) years].

Assays

Swollen and tender joints were scored as has been described (15). Blood samples were collected between 8:00 and 10:00 AM. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were determined according to standard procedures. Serum cortisol levels were determined using a fluorescence polarisation immunoassay (FPIA; Abbot, Illinois, USA) according to manufacturer instructions. The inter-assay coefficients of variation were 4.6%, 3.3% and 3.8% at serum concentrations of 0.29, 0.47 and 0.81 mmol/l, respectively (n = 54, 54 and 24).

GR density and affinity in PBMC were determined as follows: PBMC were isolated from 40 ml (EDTA) blood using Ficoll-paque density centrifugation (16-19). The cell suspension was stored overnight at 4°C in Iscove's medium supplemented with 10% foetal bovine serum that was absorbed with dextran-coated charcoal to remove free steroid (20). PBMC were centrifugated and washed 2 times with Hanks balanced salt solution HBSS (without calcium or magnesium; with 3.6 mM $NaHCO_3$, pH7.2, 4°C). Trypan blue staining revealed ≥ 95% viable cells. A binding curve was made in duplicate by adding 100 ml ^{3}H-dexamethasone in 7 concentrations (1.25-40 nM Amersham; 3.18 TBq/mmol) to 1-2 x10^6 cells per 100 µl. At the end of the incubation period (at 24°C with rotation for 90 min), cells were washed 3 times with 20 mM sodium molybdate dihydrate in HBSS to stabilise receptor-ligand binding (21), followed by quantification of the bound ^{3}H-dexamethasone using scintillation analysis. The maximum ^{3}H-dexamethasone binding based on scatchard analysis (22, 23) revealed the number of unoccupied GRs expressed in fmol/million cells, recalculated in absolute number of receptors per cell. The slope of the line in scatchard analysis reflects the GR binding affinity (Kd) expressed in nM.

Statistical evaluation

For comparison between groups, unpaired two-sided Student's T-tests or Mann-Whitney U tests were used, where appropriate. In addition, multiple regression analysis was done. Correlations, Spearman or Pearson coefficients, where appropriate, were calculated between the different parameters. Statistical significance was defined at $p < 0.05$. Data are expressed as mean ± SEM.

Results

First the results are described of the whole group (Figs.1a-c) and then those of women and men separately (Figs. 2 a-c). Figure 1a shows the serum cortisol levels of the RA patients compared with healthy controls. In this recently diagnosed RA population a statistically significantly lower serum cortisol level was found compared to the controls (35% lower, $p \leq 0.001$). Multiple regression analysis showed that age was not responsible for the difference in serum cortisol levels between the RA cohort and the healthy controls. GR receptor numbers (Fig. 1b) were also statistically significantly lower (on average almost 20 %, $p < 0.02$) in these early RA patients compared with the controls. Again, age was not responsible for this difference. Mean GR-affinity was slightly lower in the early RA patients compared with the age and sex matched control group but no statistically significant difference was obtained (Fig. 1c).

Serum cortisol levels, GR number and affinity did not correlate with age, neither in the controls, nor in the early RA group. For patients and controls together there was a positive correlation between GR number and receptor affinity (correlation coefficient 0.45, $p \leq 0.001$). No correlations of the GR parameters with serum cortisol were found.

With respect to parameters of disease activity in the RA group, there were no correlations of serum cortisol, GR-number or GR-affinity with ESR (mean 31 ± 3 mm/1st hour), CRP (mean 16 ± 3 mg/L), swollen joints (mean number 8.0 ± 0.4) or tender joints (mean number 8.7 ± 0.6).

Surprisingly, the differences between the RA patients and the healthy controls were almost entirely determined by the female patients. Significantly lower serum cortisol levels were found in the female patients compared with female controls (0.21 versus 0.41 mol/l; difference 0,20, 95% CI 0.12 - 0.28) (Fig. 2a). Between the male patients and male controls serum cortisol levels were not different. The serum cortisol levels in controls were higher (although not statistically significant) in females than in the males, but female RA patients had a lower serum cortisol level than the male patients (28% lower, $p \leq 0.02$).

Similar observations were found for the GR number. The female patients determined the difference between the RA patients and controls. A significantly lower GR-number was found in the female patients compared with female controls (7.0 versus 9.8 fmol/million cells; difference 2.8, 95% CI 1.1 – 4.6) (Fig. 2b). For males no differences were found in GR number between early RA patients and controls. The GR-number was not statistically different between male and female controls but in RA females they were lower than in the RA males (21% lower, $p \leq 0.03$).

The GR affinity showed a negative trend for both sexes:the Kd being higher means that the affinity is lower in the early RA patients compared with controls. These differences were not statistically significant (Figs. 1c and 2c).

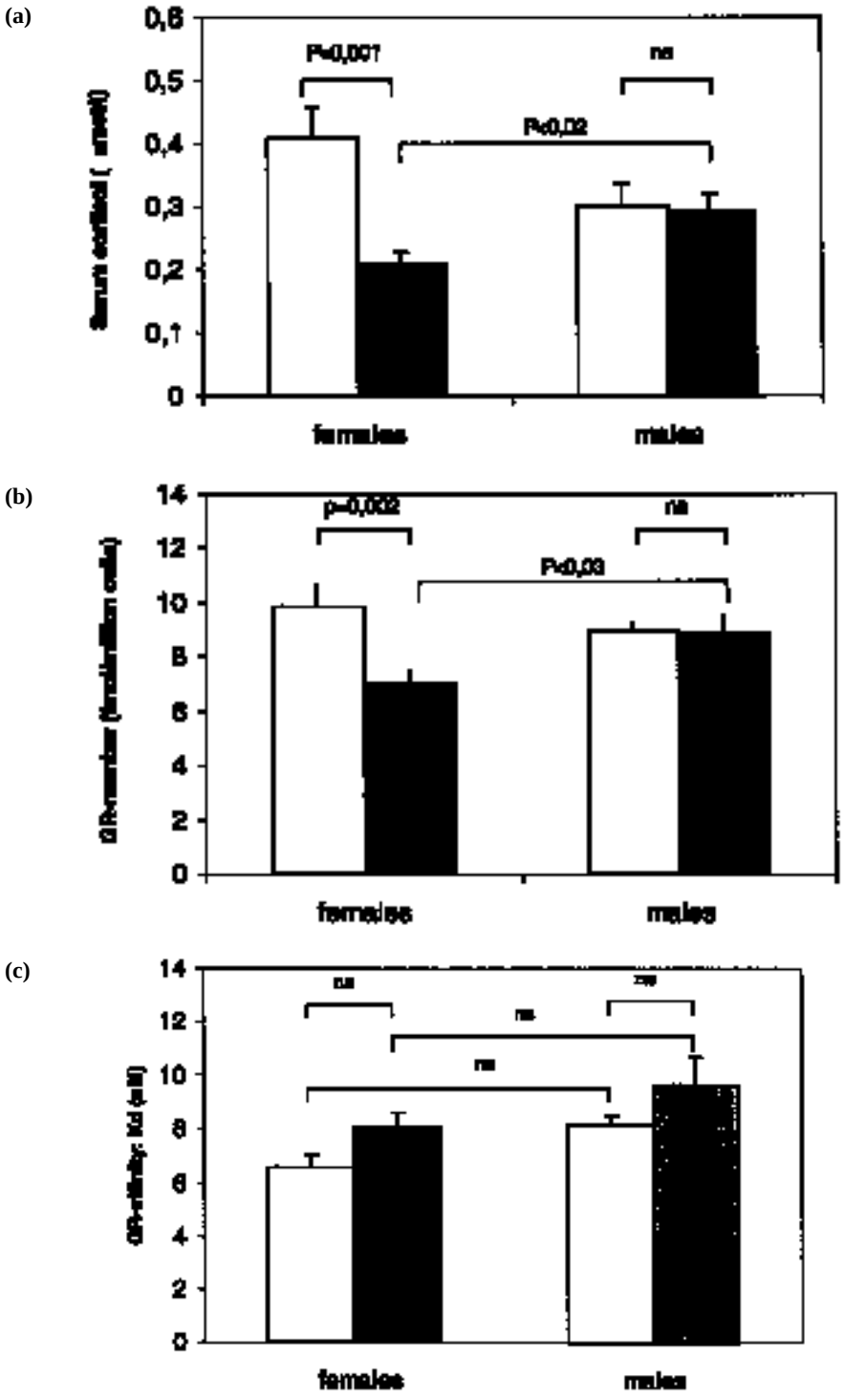

Fig. 2. **(a)** Serum cortisol levels (mol/l) of the healthy female controls (open bars) compared with female early RA group (gray bars). Mean values (SEM) for the female control group: 0.41 (0.05) and for the female early RA group 0.21 (0.02). Values for the male control group 0.30 (0.035) and for the male early RA group 0.29 (0.03).
(b) GR-number:fmol/million cells. The open bars represent the healthy control group and the gray bars the early-RA group. Mean values (SEM) for the female control group 9.84(0.79) and for the female early RA group 7.03 (0.47). Values for the male control group: 8.92 (0.35) and male early RA group 8.88 (0.71).
(c) GR-affinity (Kd) in nM. The open bars represent the healthy control group and the gray bars the early-RA group. Mean values (SEM) for the female control group 6.53 (0.44) and for the female early RA group 8.02 (0.57). Values for the male control group:8.18 (0.29) and the male early RA group 9.64 (1.02).

Discussion

In our group of early RA patients a significantly lower serum cortisol and GR number was found in the patients compared with controls. These findings suggest that glucocorticoid control of inflammation may be impaired in early RA patients. This is in agreement with the studies of Schlaghecke (adult RA patients with longer disease duration)

(6) and Andreae (children with autoimmune diseases, two thirds of them suffering from juvenile idiopathic arthritis) (24). The lower serum cortisol and GR number could completely be attributed to the female patients, since no differences were observed between the male patients and their controls. The GR-affinity tended to be lower in the patients compared with controls (Figs. 1c and 2c). However, this was not statistically significant. No correlations were found between GR-number and parameters of disease activity, nor was there a relation between GR-number and serum-cortisol levels. However, disease activity determined by ESR, CRP, painful and swollen joints was rather low, so it was less likely to find correlations.

Different studies show different outcomes with respect to early morning cortisol levels in RA (25-27). Neeck *et al.* show for active RA patients increased serum cortisol levels and disappearance of cortisol circadian rhythm (25). Their RA patients had a much higher disease activity than our early RA patients. In patients with recent onset RA the dynamic cortisol responsiveness as reflected in the early morning rise is not disturbed (27). The studies of Neeck and Dekkers are in contrast to the results of the study of van den Brink (28) and our study in which decreased morning serum cortisol levels in postmenopausal RA patients are found, reflecting a relative hypocortisolism. Above mentioned discrepancies might be attributed to differences in disease activity and disease duration, but also to the time of blood sampling, which is ideal at the moment of awakening. Patients in our study first had to travel at least an hour before blood could be sampled between 8 – 10 AM; immediate saliva sampling after awakening by the patient him or herself would have been better.

Another way to study the HPA-axis in RA might be with an overnight dexamethasone suppression test with a dose as low as 0.25 mg dexamethasone instead of 1 mg. In case of relative hypercortisolism in early RA, differences might be observed in suppressibility of the HPA-axis with an adjusted dexamethasone suppression test of 0.25 mg (29). One mg dexamethasone might be a too high dose to allow the detection of subtle individual differences in sensitivity of the HPA-axis to glucocorticoids.

In RA patients with a mean disease duration of six years, GR downregulation has been reported without change in serum cortisol levels and without correlations between GR-number and age or sex, RA-activity or serum cortisol levels (6,11). In our early female RA patients a decrease in GR number was found as well a decrease in serum cortisol levels. This was not the case in the early diagnosed male RA patients. The discrepancy with the studies of Schlaghecke might be due to the fact that the populations differ in time of onset of disease, disease activity and time of blood sampling after awakening (6, 11). The sex difference found in our study, the GR downregulation and lower serum cortisol levels being present in the early diagnosed female RA patients but not in the early diagnosed male RA patients might be related to interactions of sex hormones and the HPA-axis (2, 30, 31). The majority of patients suffering from RA are females, suggesting that the balance between androgens and estrogens plays a role in susceptibility of RA (31, 32). The depressive effects of androgens on the immune system might protect males against RA (30, 31). In addition androgens are considered as adjuvant therapy for men and postmenopausal women with RA, having anabolic and slightly disease modifying effects (33-36). Estrogens and androgens have been shown to affect the GR expression in the central nervous system (30). Estrogens decrease the expression of GRs in the anterior pituitary, hypothalamus and hippocampus of gonadectomized female rats, whereas androgens have the opposite effect upon GR expression in the medial pre-optic area of orchidectomized male rats (37-40). The possibility that similar interactions may occur in peripheral mononuclear cells opens interesting perspectives into our results, especially taking into account that male RA is associated with diminished serum androgen levels.

In conclusion, GR downregulation is not a general phenomenon; it is only present in females (especially postmenopausal women) but not in males. That makes it less likely that GR downregulation plays a major role *per se* in the aetio-pathogenesis of RA. However, it might be a co-factor determining the difference in incidence of RA, being less prevalent in males than in females and determining the difference in the severity and course of the disease and its response to glucocorticoid therapy. In addition the question arises if GR expression is different in pre versus post menopausal patients.

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