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# Glucocorticoid receptor up-regulation in early rheumatoid arthritis treated with low dose prednisone or placebo

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**ABSTRACT**

**Objective.** *Low or medium dose prednisone in early rheumatoid arthritis (RA), albeit with significant variation in clinical efficacy, reduces the progression of joint damage. The glucocorticoid receptor (GR) number in peripheral mononuclear cells (PBMC) might be helpful to predict which patients will respond to low or medium dose prednisone and therefore do not or will not need higher doses. With this in mind we determined in a double blind, placebo controlled study at baseline and yearly the GR number in PBMC.*

**Methods.** *Eighty-one early RA patients (disease duration less than one year) were included. All patients fulfilled the ACR criteria and were disease modifying antirheumatic drugs (DMARD) and glucocorticoid-naive. They were randomly assigned to treatment with 10 mg prednisone daily or placebo. From all patients disease activity (CRP, number of tender and swollen joints), the radiological joint score, bone mineral density, and the GR number in PBMC were measured annually.*

**Results.** *In females the GR number was up-regulated over time in both the prednisone and the placebo group. The same trend was observed in males. No correlations were found between the GR number in the prednisone users at the start of their treatment and changes in radiological scores or bone density after 2 years of treatment. No correlations were found between the GR number at the start and the clinical characteristics after a follow-up of 2 years.*

**Conclusion.** *The GR number in the PBMC of early RA patients did not predict which patients would be prednisone responders based on clinical or radiological parameters. However, the up-regulation of the GR number in PBMC in early RA patients towards the GR number of healthy subjects during the first two years of their disease course seems to reflect a recovery or compensatory mechanism as a response to an ongoing inflammatory process. This recovery may be not enough to efficiently control the inflammatory situation.*

## Introduction

Glucocorticoid receptors (GR) are downregulated in rheumatoid arthritis (RA), in early as well as in longstanding disease (1-3). This might reflect an impaired activity of the hypothalamic-pituitary-adrenal (HPA) axis, and thus play a role in the aetiopathogenesis of RA (4, 5). Recently, relatively low levels of ACTH and cortisol were found in relation to IL-6 and TNF in early RA compared with healthy controls (6-8). Therefore, this could be an argument to prescribe glucocorticoids to RA patients in order to compensate for the relatively insufficient HPA axis. Moreover, recent studies have shown that glucocorticoids reduce the progression of joint damage in monotherapy (10 mg prednisone) (9), as well as in combination therapy with disease-modifying anti-rheumatic drugs (DMARDs) (10-12). Biological effects of glucocorticoids at low and medium doses are mediated by the intracellular GR located in the cytoplasm of target cells (13, 14) and the number of intracellular GRs per cell is closely related to the biological response of glucocorticoids (15). Low dose prednisone is defined as 7.5 mg prednisone equivalents daily or less and a medium dose as $>7.5$ mg, but $\leq 30$ mg prednisone daily (16). Clinically, not all patients do respond in the same way: some patients respond to low doses, while others require higher doses for seemingly identical clinical situations.

The question is whether the number of GR of peripheral mononuclear cells (PBMC) might be helpful to predict which patient will respond to low or medium dose prednisone and which patient will need higher doses. In this context it is of interest to mention that in lupus patients suffering from nephrotic syndrome a distinct relationship was observed between the GR level and clinical responsiveness to glucocorticoid therapy: patients who improved or recovered had a higher GR number after glucocorticoid therapy than patients showing no improvement (17). The authors speculate that patients with a relatively higher GR number are more susceptible to the effects of glucocorticoids. Steroid-resistant asthma patients also show abnormalities in GR

expression: steroid-resistant patients exhibit a lower GR number and GR affinity than steroid-sensitive patients (18). The aim of this study was to determine the GR number at baseline and annually in the PBMC of patients being treated with 10 mg prednisone daily or placebo, the question being: Do early RA patients with a higher GR number respond better to 10 mg prednisone daily than the patients with a lower GR number?

## Material and methods

*Patients*

The study protocol was approved by the Ethics Committee of the University Medical Center Utrecht and all patients gave their informed consent. Eighty-one consecutive out-patients with recently diagnosed RA (disease duration <1 year) were included. All patients fulfilled the ACR criteria for RA and were DMARD and glucocorticoid naive. They were randomly assigned, in blocks of 10, to one of two treatment groups. One group received 10 mg prednisone daily and one group received placebo. The code of randomisation was broken after 2 years of treatment and then the prednisone dosage was tapered. Patients were allowed to use non-steroidal anti-inflammatory drugs (NSAIDs) on request. After 6 months sulfasalazine (2 gram/day) could be prescribed as rescue medication both to prednisone and placebo users. The decision to add sulfasalazine was made on clinical grounds (RA activity). Patient characteristics were as follows (Table I): 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 years). The number of male patients was high compared with female patients, with a ratio of less than 1:2. Age and sex was equally distributed over the treatment and placebo groups, as well as IgM rheumatic factor and the number of patients with erosive disease. In all patients the disease activity (CRP, number of tender and swollen joints), radiological joint score (joint erosion and joint narrowing score), bone mineral density and GR number in PBMC were measured yearly.

*Assays*

Tender and swollen joints were scored as described previously (19). The method used to score the x-rays was the van der Heijde modification of the Sharp method (20). Radiographs were taken at entry and every six months. An assistant prepared the radiographs to be read, and all identifying patient data on the radiographs were concealed from the readers. The readers had no knowledge of patients' identity when they scored the radiographs. Radiographs were read in random patient order and were scored for each patient in temporal order. Blood samples were collected between 8:00 and 10:00 AM. CRP was determined according to standard procedures.

The GR number was determined as follows. PBMC were isolated from 40 ml (EDTA) of blood using Ficoll-paque density centrifugation (21-24). 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 any free steroid (25). PBMC were centrifuged and washed 2 times with Hanks balanced salt solution HBSS (without calcium or magnesium, and with 3.6 mM $NaHCO_3$, pH 7.2, 4°C). Trypan blue staining revealed ≥ 95% viable cells. A binding curve was made in duplicate by adding 100 µl $^3$H-dexamethasone in 7 concentrations (1.25-40 nM Amersham; 3.18 TBq/mmol) to 1-2 x$10^6$ 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 the receptor-ligand binding (26), followed by quantification of the bound $^3$H-dexamethasone using scintillation analysis. The maximum $^3$H-dexamethasone binding based on scatchard analysis (27,28) revealed the number of unoccupied GRs expressed as fmol/million cells, recalculated as the 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*

All statistical analyses evaluating the effect of treatment were performed according to the intention-to-treat-principle. For the 10 patients (4 in the prednisone group and 6 in the treatment group) who withdrew during the study, the outcomes of the last measurements were carried forward, with the exception of the radiologic scores. For the comparisons between groups, unpaired two-sided Student's T-tests or Mann-Whitney U tests were used, where appropriate. Statistical significance was defined at $p < 0.05$. Data are expressed as mean ±SEM. Analyses were performed with the Number Cruncher Statistical System 97 (NCSS Statistical Software, Kaysville, Utah).

**Table I.** Baseline characteristics of the 81 patients with early RA*.

| | Prednisone n = 40 | Placebo n = 41 |
|---|---|---|
| Age in years | 60 (14) | 64 (12) |
| Male/female (n) | 17/23 | 12/29 |
| IgM rheumatoid factor positive ‡ (n) | 29 | 31 |
| Patients with erosive disease (n) # | 16 | 15 |
| Early morning stiffness in minutes | 100 (62) | 117 (71) |
| 28 Joint score for swelling | 7.3 (3.7) | 8.6 (4.3) |
| 28 Joint score for tenderness | 8.9 (5.7) | 8.6 (5.0) |
| C-reactive protein level in mg/L | 11 (18) | 20 (28) |
| Radiological score of hands and feet¥ | 11 (11) | 15 (21) |

* Number of patients (n) or means and standard deviations.
‡ RF status was considered positive when the IgM rheumatoid factor level was ≥ 25 IU/ml. This cutoff point yielded a false-positive test result for <5% of the general population.
# A Sharp-van der Heijde erosion score of ≥ 4 was considered erosive, and a score of 0 to 3 nonerosive.
¥ Erosions and joint space narrowing were assessed with the van der Heijde modification of the Sharp method.(20) Scores ranged from 0 (no damage) to 448 (maximum score for erosions and joint space narrowing in hands and feet).

## Results

In both females and males in both the treatment and placebo groups the GR number increased over time (Fig. 1). However, only in females did this increase reach statistical significance: the GR number in female prednisone users at t = 2 years versus t = 0 years was 10.2 vs. 7.5 fmol/$10^6$ cells (p using the Mann-Withney-U test = 0.043), the GR number in female placebo patients at t = 2 years versus t = 0 years was 11.0 vs. 6.7 fmol/$10^6$ cells (p using the Mann-Withney-U test = 0.000). No leucocytosis was seen after 1 or 2 years of treatment with prednisone nor a significant rise of lymphocytes or monocytes. GR affinity (Kd) after 2 years of treatment was significantly higher in the female placebo patients compared with the level at baseline. No differences in GR affinity were observed in the female prednisone users nor in the male patients.

CRP and the number of tender joints did not change significantly during the two years of the study, while the number of swollen joints decreased significantly. This illustrates that disease activity, which at t = 0 was moderate with a mean CRP of 14.2 (3.3) mg/ml in the female patients and 18.5 (4.8) mg/ml in the male patients, remained about the same during the 2-year study period. No correlations were found between the GR number in the prednisone users at t = 0 and changes in the radiological scores during the 2 years of treatment (Pearson correlation coefficient = 0.002). Nor was a correlation found with changes in BMD after 2 years (Pearson correlation coefficient for the lumbar spine –0.22; collum 0.12). However, 4 prednisone users developed osteoporotic vertebral fractures. Three of these 4 patients had very low GR numbers at t = 0, specifically 3.3, 3.3, 3.6 and 8.4 fmol/$10^6$ cells, which rose to 8.0, 8.7, 11.2 and 10.4 fmol/$10^6$ cells respectively after 1 year of treatment with 10 mg prednisone daily.

Prednisone responders, defined as the patients who did not require SASP rescue medication at t = 2 years (n = 27; 13 females,14 males) did not have a different GR number at t = 0 compared with non-responders to prednisone, i.e. patients, who needed SASP rescue medication at t = 2 years (n = 8; 6 females, 2 males).

## Discussion

The GR number in the PBMC of early RA patients did not predict which patients would be responsive to prednisone based on either clinical or radiological improvement. However, 3 out of 4 prednisone users who developed osteoporotic vertebral fractures had a very low GR number at t = 0, which was strongly up-regulated at t = 1 year. Whether this phenomenon is related to steroid-induced osteoporosis needs further investigation. This cohort of early RA patients had moderate disease activity which did not change much during the study period. However, progression of radiological joint damage was inhibited after 2 years of treatment (9). Our results might have been different in very active early RA patients with very active disease, who achieve more complete remission with higher prednisone dosages. In addition,the ratio of the isoforms of GR, GRb/GRa might play an important role in the patient's response to glucocorticoid therapy (29, 30). In this respect it also would be interesting to study cytoplasmic versus nuclear receptors and to look at the expression of NF-kB.

The GR up-regulation was present in prednisone as well as in placebo users and was most prominent in women. In female early RA patients the GR number at t = 0 is lower than the GR number in age- and sex-matched healthy controls (3). After two years the GR number of the female patients with RA was up-regulated to the GR number seen in healthy controls (3). However, this up-regulation probably was not enough to improve the inflammatory situation of the RA patient. The increase in the GR number in PBMC over time was not due to leukocytosis since in our prednisone-treated RA patients no leukocytosis was present after 1 and 2 years, nor was there a significant rise in lymphocytes or monocytes. The GR number is not influenced by age or gender (31) so the increase was not due to two years of aging in our patients either.

Up-regulation of GR seems to reflect a compensatory mechanism of the HPA-axis as a response to an ongoing inflammatory process. This is also suggested by the finding that higher serum cortisol levels with disappearance of the cortisol circadian rhythm is observed in RA patients with high activity (32). Other studies in a variety of autoimmune diseases show GR down-regulation with glucocorticoid therapy (33, 34). However, this was most obvious at higher doses than the 10 mg prednisone used daily in our study. In addition, the fact that the HPA-axis seems impaired in early RA might help explain why we did not find differences between 10 mg prednisone and placebo (35). Monotherapy with 10 mg prednisone is disease-modifying in that it results in the reduced progression of joint damage (9). Combination with another DMARD provides an even better inhibition of radiological progression (10-12). Since therapy with 10 mg prednisone produces the same up-regulation in the GR number as therapy with placebo, monotherapy with 10 mg prednisone does not seems to be able to fully suppress disease activity. This might be achieved by adding a DMARD

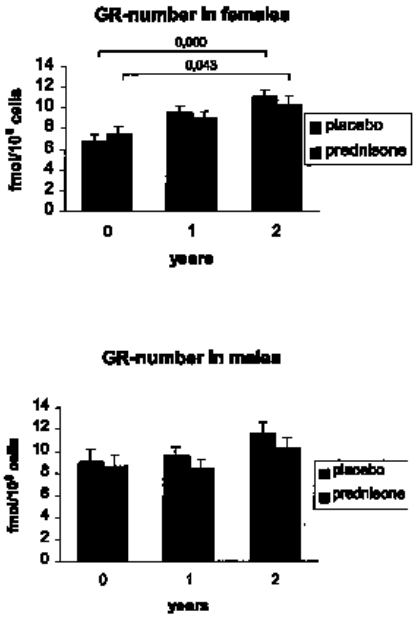


**Fig. 1.** GR number in the PBMC in female (upper panel) and male (lower panel) RA patients treated with 10 mg prednisone daily or placebo. At time (t) = 0 the patients were not yet treated. At t = 1 and t = 2 they had been treated for 1 and 2 years, respectively, with placebo or 10 mg prednisone daily.

or increasing the prednisone dose. Increasing the dose of prednisone might result in responders and non-responders, and in responders the GR number might be less suppressed than in non-responders.

In conclusion, this is the first study to follow the change in GR number in the PBMC of early RA patients being treated with 10 mg prednisone daily compared to placebo. GR up-regulation seems to reflect a recovery or compensatory mechanism in response to an ongoing inflammatory process. This recovery may be not enough to counteract the inflammatory situation at that moment, despite the fact that the progression of radiological damage is inhibited by 10 mg prednisone daily. The GR number in the PBMC of early RA patients did not predict which patients were going to be prednisone responders clinically or radiologically.

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