Relation of plasma dexamethasone to clinical response

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Abstract
Objective
The clinical effects of high dosage pulse glucocorticosteroid (GS) infusion as a treatment for rheumatoid arthritis (RA) differ considerably between patients. The aim of the present study was to gain more insight into these differences in clinical response.

Methods
Twenty-three RA patients (6 M/ 17 F) with treatment-resistant active erosive disease were treated with GS pulse therapy, consisting of 3 infusions of 200 mg dexamethasone at 3-day intervals. Plasma dexamethasone and plasma cortisol levels, as well as the mononuclear cell glucocorticosteroid receptor density, were determined on days 0, 2, 6, 12 and 40 after the start of therapy. Clinical evaluation consisted of the Thompson articular index, the erythrocyte sedimentation rate (ESR), and the serum concentration of C reactive protein (CRP).

Results
Plasma dexamethasone levels in RA patients determined during pulse therapy revealed the existence of two groups. One group reached significantly (p < 0.05) higher plasma levels than another group comparable for age and sex. The CRP, ESR and Thompson joint score prior to the start of pulse therapy were all higher (p < 0.05) for the high plasma dexamethasone group. The decrease in ESR, CRP and the Thompson joint score was also significantly greater (all p < 0.05) for the high plasma dexamethasone group. Plasma cortisol, as well as the GS receptor density at the start of treatment, did not differ between the two groups; both decreased after the first pulse in both groups and returned to pre-treatment values shortly after the last infusion.

Conclusion
The treatment of refractory RA with dexamethasone pulse therapy is, on average, beneficial. The high plasma dexamethasone levels reached might depend on the greater severity of the disease in these patients prior to the start of the treatment, and result in greater changes in the disease parameters. Glucocorticosteroid receptor density measurements made during and directly after high dose pulse dexamethasone treatment proved to be unreliable because of the high plasma dexamethasone levels.

Key words
Rheumatoid arthritis, glucocorticosteroid, dexamethasone.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown aetiology. Its most characteristic feature is a persistent inflammatory synovitis, which usually involves the peripheral joints in a symmetrical fashion. The articular inflammation may be remittent but is usually chronic, causing destruction of the cartilage and erosion of adjacent bones. Various extra-articular manifestations, such as vasculitis, neuropathy, scleritis, pericarditis, lymphadenopathy and splenomegaly, are quite common. These manifestations are integral features of the disease and illustrate its systemic nature (1).

Glucocorticosteroids (GS) have been used to treat patients with active RA for more than 50 years now (2). The precise mechanism of action of GS therapy is only partly understood (3, 4). GS have direct and indirect effects on the immune system. Direct effects are observed on nearly all types of inflammatory cells. GS influence the distribution of circulating lymphocytes and may depress the adhesion molecules on lymphocytes. They inhibit several events associated with T-cell activation, such as the production of cytokines and they inhibit the function of all T-cells: helper and suppressor as well cytotoxic T-cells. GS influence B-cells only when given in high dosages; then they may decrease serum immunoglobulin synthesis. GS antagonise macrophage differentiation and inhibit many of their functions (5). Apart from the immune cells, other mechanisms relevant for inflammation are also influenced by GS, e.g. the formation of arachidonic acid metabolites (6). In summary, lower dosages of GS inhibit leucocyte traffic and the cellular immune response, whereas higher dosages are required to suppress the functions of leucocytes and the humoral immune response. Apart from this dosage response, there is also a heterogeneity of response among different persons with rheumatoid arthritis. This heterogeneity is not well understood.

The indirect effects of GS are mediated by the hypothalamic-pituitary-adrenal axis (7). The hypothalamic-pituitary-adrenal axis plays a crucial role in maintaining homeostasis, including the regulation of inflammation. Pro-inflammatory cytokines may affect the production of cortisol induced by the axis, which in turn suppresses inflammation, giving a negative feedback signal. Some studies suggest adrenal insufficiency and a disturbed circadian rhythm of plasma cortisol levels in RA (8). Such findings suggest that the regulation of cortisol levels to modulate inflammation might be insufficient in patients with RA (9). On the other hand, the magnitude of the biologic effects of GS may depend on the density and affinity of the GS receptors on the target cells (10). It has been reported that the peripheral blood mononuclear cells of patients with RA have a markedly lower receptor density than those of healthy controls (11).

The long-term treatment of RA with relatively high dosages of GS, 20 mg/d or more, has been disappointing because of incomplete responses, severe complications or, quite often, both. Accordingly, regimens have been modified to improve the therapeutic index of higher GS dosages. One such an approach is the use of intravenous pulse therapy. The infusion of high doses of GS - for example methylprednisolone up to $1.0 \text{ g/m}^2/\text{d}$ for 1-5 days - has been used in different rheumatic diseases (12). This treatment, which is often called pulse therapy, is administered in cases of treatment-resistant crippling RA, to bridge the period before the onset of action of slow-acting, disease-modifying agents, and in cases of life-threatening vasculitis or other serious extra-articular symptoms, often in combination with a cytotoxic agent. Thus, pulse therapy should not be used on its own but only as part of an overall strategy in the treatment of the individual patient (13).

Such high doses administered over a relatively short time may have specific therapeutic effects on the immune system. Interestingly, the beneficial effects of high dose GS pulse therapy on disease activity and laboratory parameters differ considerably between patients. The aim of the present study was to gain information on the differences in response to this pulse therapy in patients with RA. We therefore investigated whether the actual plasma dexamethasone levels reached, the GS receptor expression and/

or the plasma cortisol levels are related to the clinical effect.

Patients and methods

Patients

Patients (n = 23) admitted to hospital for GS pulse therapy were evaluated. All patients fulfilled the ACR criteria for RA and had treatment-resistant active, erosive disease which could not be controlled by treatment with disease-modifying antirheumatic drugs (DMARDs) only. Patients were only admitted to the study if 4 weeks prior to the start of the pulse therapy no oral or intra-articular GS had been taken.

The GS pulse protocol consisted of 3 infusions of 200 mg dexamethasone at 3-day intervals (day 0, 3 and 6). Blood samples were taken and a clinical evaluation was performed just before and 2, 6, 12 and 40 days after the first infusion, between 8:00 and 9:00 A.M. On day 6 this was done before the third infusion. Disease activity was scored using the Thompson articular index (14), the erythrocyte sedimentation rate (ESR; Westergreen method) and the serum concentration of C-reactive protein (nephelometric).

Assays

Plasma dexamethasone levels were determined using a radioimmunoassay described by Thijssen (15). Plasma cortisol levels were determined using a fluorescence polarization immunoassay (FPIA; Abbott, Illinois, USA). 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 μM, respectively (n = 54, 54 and 24). The levels of cortisol measured in the presence of extemely high dexametasone levels resulted in a cross reactivity of 0.36% (dexamethasone level 127 μM).

The GS receptor density of peripheral blood mononuclear cells (PB MNC) was determined according to Steiner (16). PB MNC were isolated from 40 ml EDTA blood. Viability was checked by Trypan Blue exclusion and always exceeded 95%. A binding curve was made by adding 100 μl ^3H-dexamethasone in 7 concentrations (1.25 - 40 nM; Amersham, UK; 3.18 TBq/mmol) to 2 x 10^6 cells per 100 μl. At the end of the incubation period (duplicate at 24°C for 90 min), cells were washed 3 times with 20 mM sodium molybdate dihydrate in Hanks' balanced salt solution (without calcium

or magnesium; with 3.6 mM NaHCO3, pH 7.2; 4°C) to stabilize receptor-ligand binding, followed by quantification of the bound ^3H-dexamethasone using scintillation analysis. Scatchard analysis revealed the number of unoccupied GS receptors. To confirm the specificity of the assay, competition experiments were performed with radio-inert hydrocortisone, progesterone, oestradiol and testosterone. More than 20, 100, 1000 and > 10,000 fold concentrations were needed to obtain similar binding as obtained with dexamethasone, respectively.

Statistical evaluation

Unpaired non-parametric statistical evaluation (Mann-Whitney U) was used for the comparison between groups (mean values ± SEM are given, n = 6). For the statistical evaluation of changes within groups paired non-parametric analyses (Wilcoxon) were used. Statistical significance was accepted at P < 0.05.

Results

Plasma levels of dexamethasone and cortisol

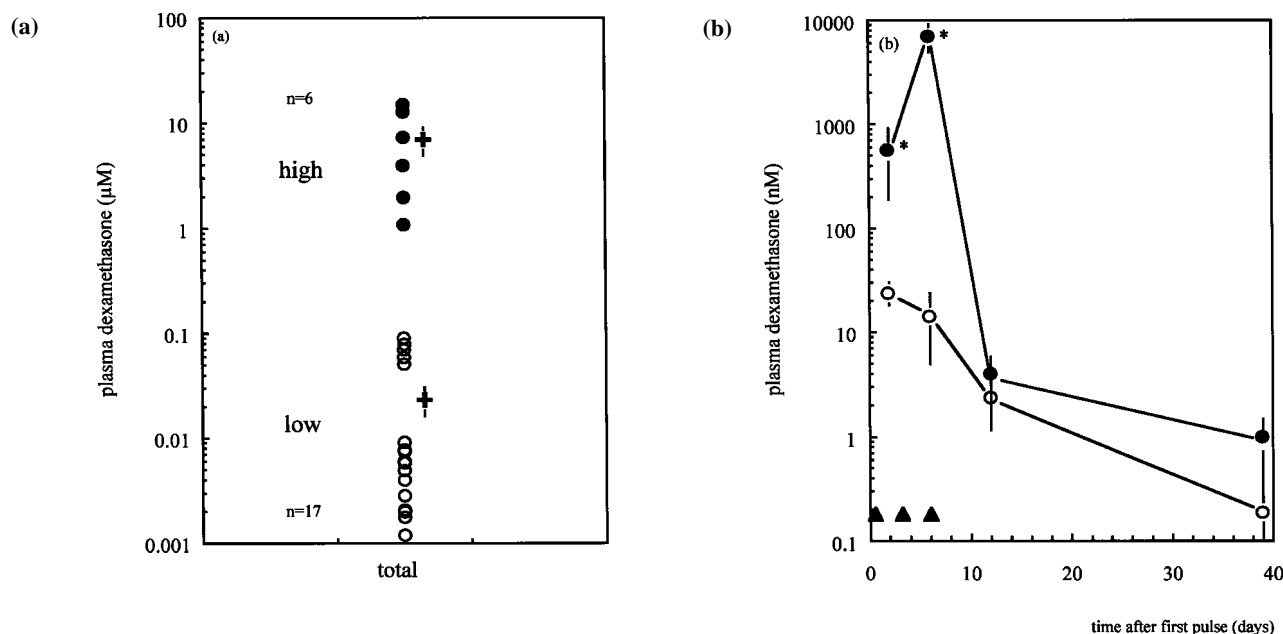Plasma dexamethasone levels in the RA patients determined during the pulse

Fig. 1. (a) Plasma dexamethasone levels in 23 RA patients just before the 3rd infusion on day 6 after the start of pulse therapy, comprising dexamethasone infusions at days 0, 3, and 6. A statistically significant dichotomy in plasma dexamethasone levels was noted.
(b) Plasma dexamethasone levels during pulse therapy in patients who reached extremely high plasma dexamethasone levels, and in a group of age- and gender-comparable patients reaching relatively low plasma dexamethasone levels. Mean values ± SEM (n = 6) are given. Asterisks indicate statistically significant differences between both groups. ▲ indicate the time of dexamethasone infusions on days 0, 3 and 6, respectively.

Table I. Demographics and disease parameters before start of high dose dexamethasone pulse treatment.

Plasma dexamethasone	High (n = 6)			Low matched (n = 6)			Low total (n = 17)	
	mean	(SEM)	p	mean	(SEM)	p	mean	(SEM)
Age (years)	57.3	(6.4)	ns	57.2	(5.8)	ns	57.1	(6.3)
Sex M/F	2/4		ns	2/4		ns	4/13	
Disease duration (years)	8.2	(3.2)	ns	8.3	(2.4)	ns	10.4	(2.3)
Rheumatoid factor +/-	6/0		ns	6/0		ns	16/1	
Cortisol (μM)	0.59	(0.10)	ns	0.50	(0.06)	ns	0.60	(0.11)
ESR (mm/h)	91.2	(14.8)	< 0.05	45.8	(11.2)	ns	63.3	(8.1)
CRP (mg/l)	84.4	(19.4)	< 0.05	32.3	(13.1)	ns	52.4	(10.8)
Thompson index	430	(48)	< 0.05	241	(54)	ns	267	(38)

Classification as high or low plasma dexamethasone is based on the dichotomy shown in Figure 1. From the group with relatively low plasma dexamethasone levels, a group (matched for age and sex) was selected to be compared to the group with the extremely high plasma dexamethasone levels. Mean values ± SEM are given for the high (n = 6), low matched (n = 6), and total low (n = 17) groups, respectively. ns: difference not statistically significant.

therapy revealed the existence of two clusters. Six patients reached significantly higher plasma levels than the other patients (Fig. 1a). For these 6 patients (aged 57.3 ± 6.4 years, M/F 2/4), an age- and sex-comparable group was selected from the group of patients with relatively low plasma dexamethasone levels. With respect to age, sex ratio, disease duration and disease activity there were no statistically significant differences between the matched and total groups with low plasma dexamethasone levels (Table I).

Plasma dexamethasone levels during and after pulse therapy for the group reaching high plasma dexamethasone levels

and for the age- and sex-comparable group reaching relatively low plasma dexamethasone levels are shown in Figure 1b. The high plasma dexamethasone levels reached during the pulse declined in both groups quickly after the last infusion.

The plasma cortisol levels of the age- and sex-comparable groups with high and low plasma dexamethasone levels are depicted in Figure 2. Cortisol levels before the start of the pulse did not differ between the two groups; nor did they differ between the selected group and the whole group with low plasma dexamethasone levels (Table I). Surprisingly, plasma cortisol levels were statistically

significantly less suppressed in the group with high plasma dexamethasone levels than in the comparable group with low plasma dexamethasone levels.

Disease parameters
The CRP, ESR and Thompson articular index prior to the start of the pulse therapy for the three groups of patients are shown in Table I. All three parameters were higher for the high plasma dexamethasone group than for the age- and sex-comparable low plasma dexamethasone group. Disease parameters did not differ statistically between the comparable and the total groups with low plasma dexamethasone.

The effect of dexamethasone pulse therapy on the ESR, CRP and Thompson articular index are shown in Figures 3a, 3b and 3c, respectively. The changes in ESR, CRP and Thompson articular index were statistically significantly greater for the high plasma dexamethasone group compared to the low plasma dexamethasone group. At day 40, the effects on the CRP, ESR and Thompson index persisted in the high plasma dexamethasone group but not in the low plasma dexamethasone group.

Glucocorticosteroid receptor assay
The GS receptor density at the start of treatment did not differ between the two groups, as shown in Figure 4. The unoccupied GS receptor density decreased after the first pulse in both groups. However, during treatment the decrease was stronger and more prolonged in the
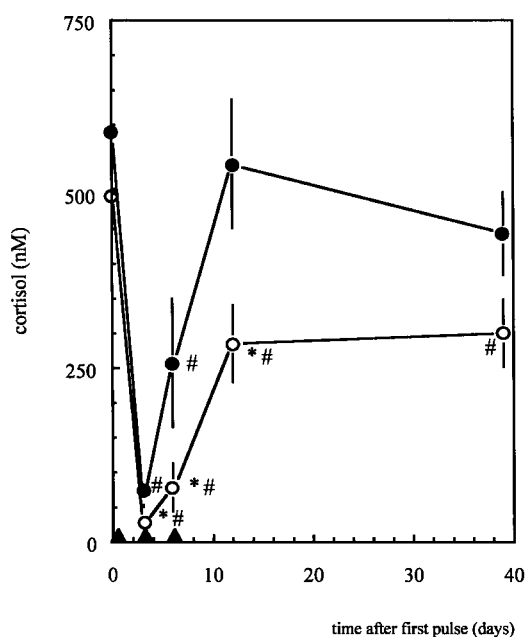

Fig. 2. Plasma cortisol levels before, during and after the pulse therapy of patients who reached high plasma dexamethasone levels (●) and of age- and sex-comparable patients reaching relatively low plasma dexamethasone levels (○). Mean values ± SEM (n = 6) are given.
indicate statistically significant changes compared to the values before the start of pulse therapy; * indicate statistically significant differences between both groups. ▲ indicate the time of the infusions of dexamethasone on days 0, 3 and 6, respectively.
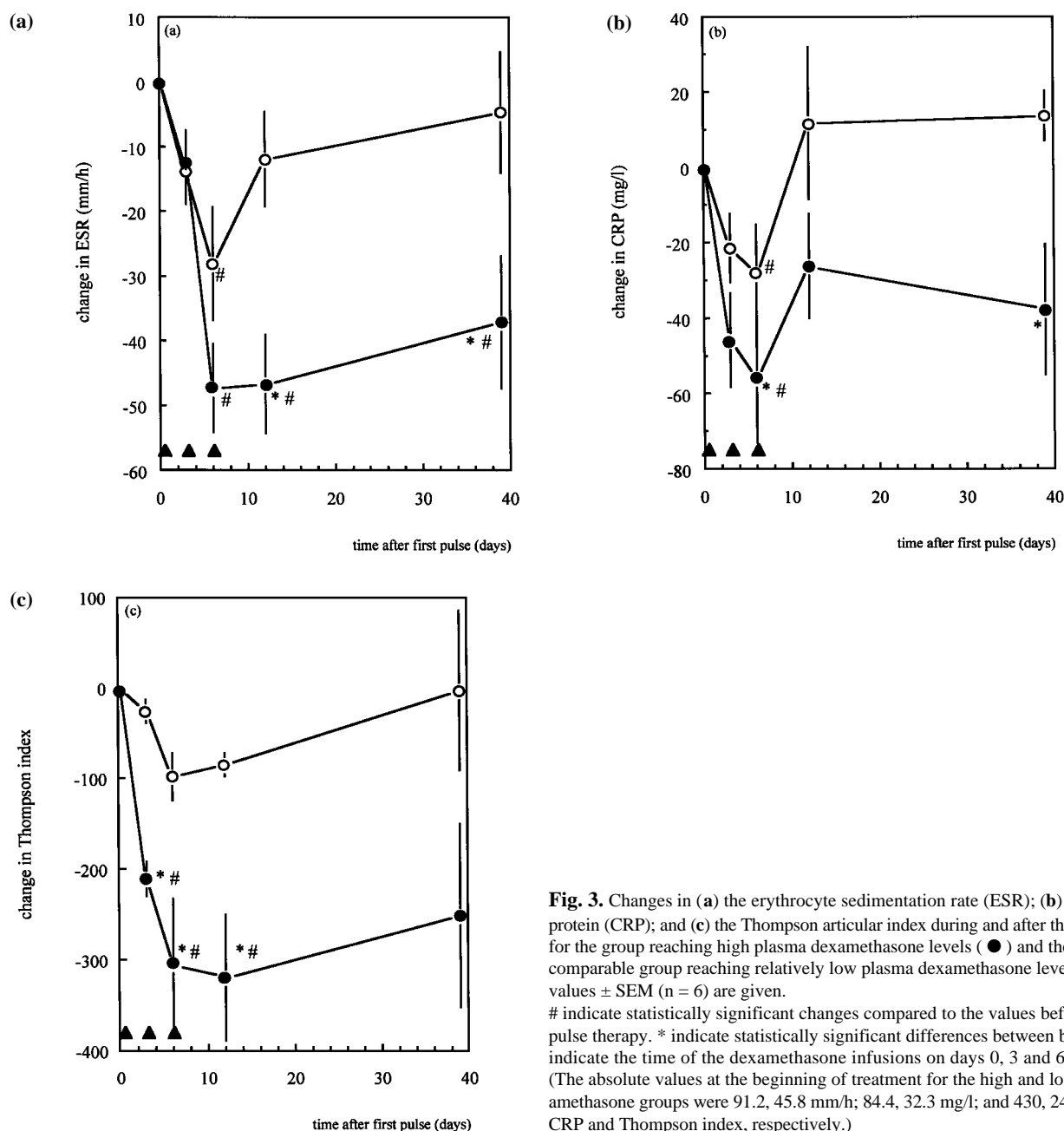
(a)

(b)

(c)

Fig. 3. Changes in (**a**) the erythrocyte sedimentation rate (ESR); (**b**) the C-reactive protein (CRP); and (**c**) the Thompson articular index during and after the pulse therapy for the group reaching high plasma dexamethasone levels (●) and the age- and sex-comparable group reaching relatively low plasma dexamethasone levels (○). Mean values ± SEM (n = 6) are given.
indicate statistically significant changes compared to the values before the start of pulse therapy. * indicate statistically significant differences between both groups. ▲ indicate the time of the dexamethasone infusions on days 0, 3 and 6, respectively. (The absolute values at the beginning of treatment for the high and low plasma dexamethasone groups were 91.2, 45.8 mm/h; 84.4, 32.3 mg/l; and 430, 241 for the ESR, CRP and Thompson index, respectively.)

group which reached high plasma dexamethasone levels compared to the comparable group with low plasma dexamethasone levels which appeared to be refractory to further administration of dexamethasone. GS receptor levels returned to pre-treatment values shortly after the last infusion with dexamethasone.

Although most of the plasma dexamethasone is washed out during PB MNC isolation, the measured decrease in unoccupied GS receptor density of the isolated cells could be the result of remaining dexamethasone, especially at the high plasma dexamethasone levels. The dexamethasone concentration after cell isolation compared to before cell isolation in the incubation mixture was 0.10 ± 0.05%, as determined by RIA or using ^{3}H-dexamethasone as a tracer.

Whether the remaining 0.1% dexamethasone could be responsible for the decreased receptor level was examined as follows. Blood from two pulse patients, obtained before (no plasma dexamethasone) and at day 6 after the start of the pulse (7.0 µM and 3.5 µM plasma dex-

amethasone for patients X and Y, respectively; Fig. 5a). GS receptor density was determined in cells isolated from these patients (Fig. 5a). Additionally, the plasma of these patients was added to the isolated cells of healthy controls for 60 min at 37°C, after which the GS receptor density was determined (Fig. 5b). It was found that the GS receptor density before, compared to during the pulse, decreased to the same extent for both the patients' cells and for the control cells mixed with patients' plasma (81% vs 80% and 39 %vs 36%, for patients X and
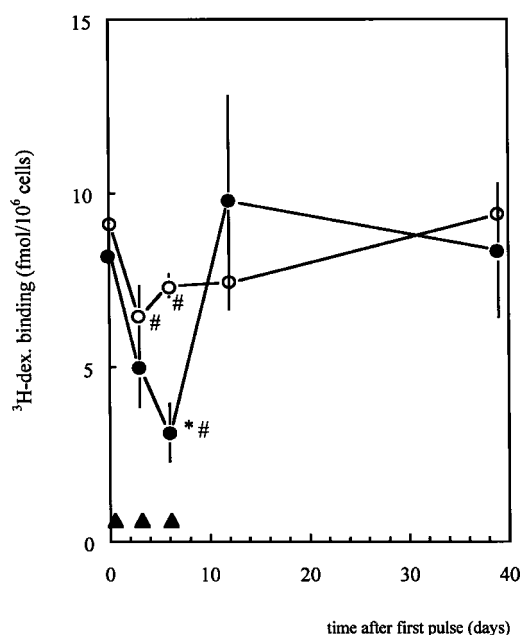
Fig. 4. ^{3}H-dexamethasone binding representing the unoccupied receptor quantity (fmol), expressed per million cells before, during and after pulse therapy for both the group which reached extremely high plasma dexamethasone levels (●) and the age- and sex-comparable group which reached relatively low plasma dexamethasone levels (○).
Mean values ± SEM (n = 6) are given.
indicate statistically significant changes compared to the values before the start of pulse therapy.
* Indicates a statistically significant difference between both groups.
▲ indicate the time of dexamethasone infusions on days 0, 3 and 6, respectively.

Y, respectively). This indicates that the remaining 0.1% dexamethasone indeed interfered with the GS receptor assay.

In an additional experiment, plasma dexamethasone levels were mimicked *in vitro* by adding 2.5, 10, 25 and 250 nM dexamethasone (levels determined during the pulse, see Fig. 1b) to whole blood for 60 min at 37°C. It was found that at 2.5 nM dexamethasone and higher, the receptor density decreased. At 10 nM dexamethasone, the receptor density was decreased by more than 35%, which was statistically significant. At higher dexamethasone concentrations, receptor determination became technically impos-sible because the high amounts of dexamethasone disturbed the scatchard analysis and no straight lines could be obtained (the correlation coefficients for linear regression were 0.79 and 0.41 for 25 and 250 nM, respectively). Surprisingly, the GS receptor density could be properly determined when these concentrations of dexamethasone (25 and 250 nM) were measured in the plasma as a result of the pulse therapy.

Discussion

The present results confirm that the treatment of refractory rheumatoid arthritis (RA) with dexamethasone pulse therapy is, on average, beneficial. For all the patients tested decreases in the ESR, CRP and Thompson articular index were observed (12).

Surprisingly, upon determination of the plasma dexamethasone levels a dichotomy appeared. There was a group that reached extremely high plasma dexamethasone levels and a group which reached significantly lower plasma dexamethasone levels. This dichotomy was also present with respect to changes in the disease parameters induced by the treatment. The decrease in the CRP, ESR and Thompson index was significantly stronger for the group which reached high plasma dexamethasone levels compared to the group which reached relatively low plasma dexamethasone levels.

It is tempting to speculate that the degree of change in the disease parameters depends on the plasma dexamethasone levels reached, high plasma dexamethasone levels corresponding with the most profound changes. However, those patients reaching high plasma dexamethasone levels had significantly higher CRP, ESR and Thompson articular index values prior to the start of treatment compared to the patients who reached low plasma dexamethasone levels. The plasma dexamethasone levels reached may therefore depend on the severity of the disease before the start of the dexamethasone infusion.

Active RA is associated with numerous signs of inflammation, such as changes in the cytokine profile, adhesion molecules, enzymes, products of arachidonic acid metabolism and so on. On these and other levels there might be an interference between the three parameters of disease and the levels of dexamethasone reached. The suggestion that the level of dexamethasone reached depends on the disease activity of the RA patients is supported by the fact that a dichotomy in the plasma dexamethasone levels reached was not found upon treatment of healthy volunteers (20). It is relevant to note that the clinical effect is in line with the plasma dexamethasone concentrations reached. This also confirms the clinical findings that pulse therapy with intravenous GS is more effective when given in a higher dosage and for 3 infu-
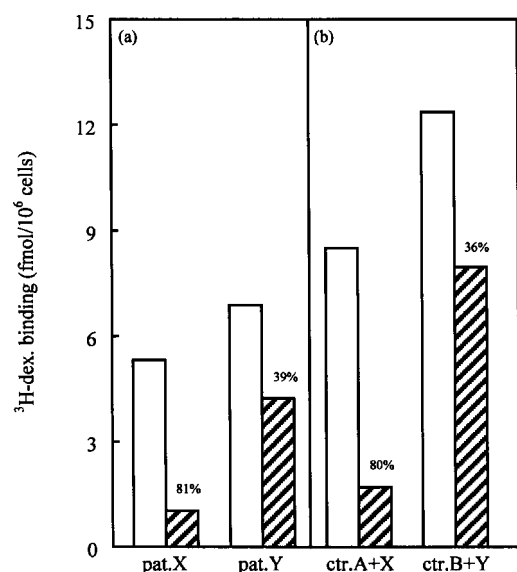

Fig. 5. ^{3}H-dexamethasone binding in mononuclear cells as a measure of GS receptor density. Comparison between the effect of dexamethasone pulse therapy in two RA patients (X and Y; **Panel a**) and GS receptor density in isolated cells of healthy controls to which plasma of the patients from panel a was added. Measurements were made before the start of pulse therapy (**open bars**) and just before the third dexamethasone infusion (**hatched bars**). During the dexamethasone pulse therapy, the GS receptor density decreased by 81% and 39% for both patients, respectively. The plasma of these two patients added to PB MNC isolated from healthy controls resulted in similar inhibitions (80% and 36%, respectively). The mean values for duplicate samples are given.

sions at a time (9).

There appeared to be a relationship between the plasma dexamethasone levels and the GS receptor density following dexamethasone administration; high dexamethasone levels were associated with low GS receptor densities. However, this observation actually originated from an artefact, i.e. the residual amount of dexamethasone left after cell isolation. For plasma concentrations of 2.5 nM and higher, the dexamethasone remaining after cell isolation appeared to interfere with the receptor assay, decreasing the number of measurable unoccupied receptors. This implies that during and immediately after pulse therapy, the GS receptor density cannot be determined reliably (17). Since the GS receptor level measured before the start of the treatment has no predictive value with respect to the treatment outcome, we may therefore conclude that the measurement of GS receptor density in relation to high dose dexamethasone treatment is of no value.

Interestingly, the concentrations of dexamethasone 25 nM and upwards added *in vitro* to whole blood were so high that a proper determination of the GS receptor density became impossible. Such high amounts of dexamethasone were only detected in patients reaching high plasma dexamethasone levels at days 2 and 6 after the start of the pulse. However, in these patients the GS receptor density could be determined properly at these time points. Moreover, by contrast in blood samples taken directly after pulse administration the GS receptor density could not properly be determined (data not shown). This suggests that the dexamethasone as measured was (in part) incompetent to bind to the receptor. This could indicate the formation of metabolites which are "detected" as dexamethasone in the immunoassay, but which are unable to bind to the GS receptor and as a consequence do not disturb the receptor assay.

Keto-metabolites are usually formed after dexamethasone administration. 17-Keto-dexamethasone (a generous gift from Organon, Oss, The Netherlands) gave in a dexamethasone radioimmunoassay a cross reactivity of 0.1% at a concentration of 3 μM (data not shown).

Therefore, the possible formation of this metabolite could not explain our results. Nevertheless, the formation of metabolites other than 17-keto-dexamethasone is corroborated by the finding that cortisol levels correlated positively with the plasma dexamethasone levels (r = 0.49 p < 0.05, n = 24) when the extremely high plasma dexamethasone levels (2 and 6 days after the first infusion) were taken (compare Figures 1b and 2). However, the whole group plasma dexamethasone levels correlated inversely, as expected (18), with the plasma cortisol levels (p = 0.0027, n = 48).

The cross reactivity of dexamethasone in the cortisol immunoassay is reported to be 0.36% and therefore may not be the explanation for the high cortisol values found in the group of patients which reached a high plasma dexamethasone level. Dexamethasone does not bind to transcortine (GS binding globuline) and therefore does not explain the dichotomy (19). In addition, assuming a half-life of 3.5 hours (20), the calculated levels at days 2 and 6 after the start of pulse therapy should not exceed 2 nM and 2 pM, respectively, whereas 300 nM and 3500 nM on average were measured, respectively. Why dexamethasone metabolites accumulate only in some patients, resulting in high plasma immunoreactive dexamethasone levels, remains unclear. No relationship with liver insufficiency was found.

In conclusion, the present study has demonstrated that the treatment of refractory RA with dexamethasone pulse therapy is, on average, beneficial. A dichotomy was noted, showing patients who reached a high plasma dexamethasone level and others who reached a less high dexamethasone level. The high plasma dexamethasone levels reached might depend on the greater severity of the disease prior to the start of the treatment in these patients, and might have resulted in the more profound changes in disease parameters seen. GS receptor density measurements taken during and directly after the high dose pulse dexamethasone treatment proved to be unreliable. The high dexamethasone levels measured probably depend on the formation of metabolites ineffective to bind to the GS receptor.

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