Glucocorticoid sensitivity in multiple sclerosis; what makes the difference?

van Winsen, L.M.L.

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Chapter 3

Clinical implications of differences in GC sensitivity

Chapter 3.1

In vitro suppressive effect of glucocorticoids on TNF-α production is associated with their clinical effect in multiple sclerosis

L.M.L. van Winsen, C.H. Polman, C.D. Dijkstra , F.J.H. Tilders and B.M.J. Uitdehaag

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Abstract

GCs can inhibit the production of pro-inflammatory cytokines like tumor necrosis factor α (TNF-α), which may be one of the relevant effects of treatment with high dose IVMP. We prospectively studied 27 MS patients who were treated with IVMP. Before and after treatment *in vitro* stimulated TNF-α production in blood cells and the effect of *in vitro* administered GC were determined. The suppression of TNF-α production after IVMP, and the *in vitro* suppressive effect of GC prior to treatment was related to subsequent clinical improvement after IVMP. The results suggest the existence of a partial GC resistance, in a subgroup of MS patients, which may have implications for treatment efficacy.

Introduction

High dose IVMP is often used to treat worsening of symptoms in MS (30). However, not all patients show an equally positive clinical response to this GC treatment and the underlying mechanism responsible for these interindividual differences is unknown. Recently, we described the existence of a partial GC resistance in MS (75). The clinical implications are not yet fully understood.

Various biological mechanisms of GC have been assumed to be relevant in the proposed pathogenesis of MS (46, 114, 115). Methylprednisolone (MP) affects a wide range of biological mechanisms, anti-inflammatory and immunomodulatory effects being especially pronounced (40, 116). This includes the suppression of pro-inflammatory cytokines, like TNF-α. We hypothesised that the suppressive effect of GC on the production of *in vitro* LPS stimulated TNF-α production by PBC can be used as a measure for GC sensitivity, and that this is related to its clinical effect.

To test this hypothesis, we prospectively studied 27 MS patients who were treated with a short course of high dose IVMP.

Patients and Methods

We prospectively selected 27 MS patients scheduled to undergo treatment with IVMP (11 men and 16 women) in such a way that patients with relapsing remitting (RR) secondary progressive (SP) and primary progressive (PP) disease were equally represented. The study was approved by the ethics committee of our hospital and all patients gave informed consent to undergo all study procedures.

Treatment consisted of 1000 mg IVMP daily for 3 consecutive days in 22 patients or 500 mg IVMP daily for 5 consecutive days. If treated with steroids previously, the interval was at least 4 months and in 13 of the 15 patients it was 6 months or more.

The clinical response to treatment was evaluated after 6 weeks. A significant improvement was defined as decrease on the EDSS from baseline of 1.0 point or more (for those with EDSS scores < 6.0) or of 0.5 (for those with EDSS scores > 5.5). In all other cases it was concluded that there was no improvement.

Blood was drawn on the morning of the first day, before the start of the treatment (PRE-MP) and after a cumulative dose of 2000 mg MP (thus in the morning on day 3 or 5; POST-MP). The effect of *in vivo* MP was evaluated before and after treatment. PBC were stimulated by adding LPS using a whole blood assay previously described (75). The plasma was removed and stored at –20 °C until TNF-α analysis was performed in one batch using a commercially available ELISA-kit for human TNF-α (CLB, Amsterdam, The Netherlands).

The suppressive effect of GC *in vitro* was determined only before treatment by adding dexamethasone (DEX) or MP in different concentrations simultaneously with the LPS and expressed as the percentage decrease compared to TNF-α production in the absence of GC.

For statistical evaluation of differences in TNF-α production and suppression by GC between subgroups either t-tests or Mann-Whitney U test (in case of non-normal distribution) and the Fisher's exact test were used.

Results

Of the 9 RRMS patients (6 female, mean age 38.2 ± 11.9 years, mean disease duration 7.2 ± 5.2 years, mean EDSS 4.2 ± 1.3) four showed a significant clinical improvement. Of the 9 SP MS patients (7 female, mean age 46.3 ± 9.4 years, mean disease duration 8.1 ± 4.6 years, mean EDSS 6.4 ± 0.4) five showed a significant clinical improvement, and this was the case in only two of the nine PPMS patients (3 female, mean age 45.6 ± 11.7 years, mean disease duration 7.6 ± 3.9 years, mean EDSS 4.6 ± 1.6).

In all patients stimulation with 10 μg / ml LPS resulted in a higher production of TNF-α than stimulation with 5 μg / ml. There were no significant differences in stimulated production of TNF-α between RR, SP and PP patients (table). Likewise, no association was found between TNF-α production and age, sex, disease duration, EDSS score or history of previous steroid treatment. After treatment with IVMP, LPS induced production of TNF-α was strongly reduced (table). In patients showing a clinical improvement, the suppression of TNF-α production was higher than in patients without clinical response to treatment (Mann Whitney U test, p=0.021).

Since there was no significant difference between subgroups, we pooled the patients to evaluate the relation between the *in vitro* suppressive effect of GC before treatment and the clinical effect of IVMP. This suppressive effect was higher in patients who showed clinical treatment response compared to those who did not (Mann-Whitney U test, p=0.007; figure).

Table. *In vitro* production of TNF-α before and after IVMP treatment and the *in vitro* effect of corticosteroids. Values represent TNF-α concentration in ng/ml medium and are expressed as mean ± standard deviation

	all patients (n=27)	RR (n=9)	SP (n=9)	PP (n=9)
PRE-MP				
5 μg/ml LPS	2.1 ± 1.6	1.9 ± 1.7	1.8 ± 1.4	2.5 ± 1.7
10 μg/ml LPS	3.1 ± 2.1	2.8 ± 2.2	3.0 ± 2.0	3.6 ± 2.1
10 μg/ml LPS + DEX-7M	2.9 ± 1.9	2.9 ± 2.4	2.8 ± 1.8	3.2 ± 1.7
10 μg/ml LPS + DEX-6M	1.7 ± 1.3	1.4 ± 1.3	1.7 ± 1.3	2.1 ± 1.3
10 μg/ml LPS + MP-6M	2.0 ± 1.6	1.8 ± 1.6	1.7 ± 1.4	2.5 ± 1.8
10 μg/ml LPS + MP-5M	0.7 ± 0.7	0.7 ± 0.8	0.7 ± 0.5	0.8 ± 0.9
POST-MP				
5 μg/ml LPS	0.3 ± 0.3	0.2 ± 0.3	0.2 ± 0.3	0.3 ± 0.4
10 μg/ml LPS	0.5 ± 0.5	0.4 ± 0.6	0.4 ± 0.5	0.7 ± 0.6

TNF-α = tumor necrosis factor α; MP = methylprednisolone; DEX = dexamethasone;
LPS = lipopolysaccharide; PRE-MP = before intravenous MP treatment;
POST-MP = after intravenous MP treatment

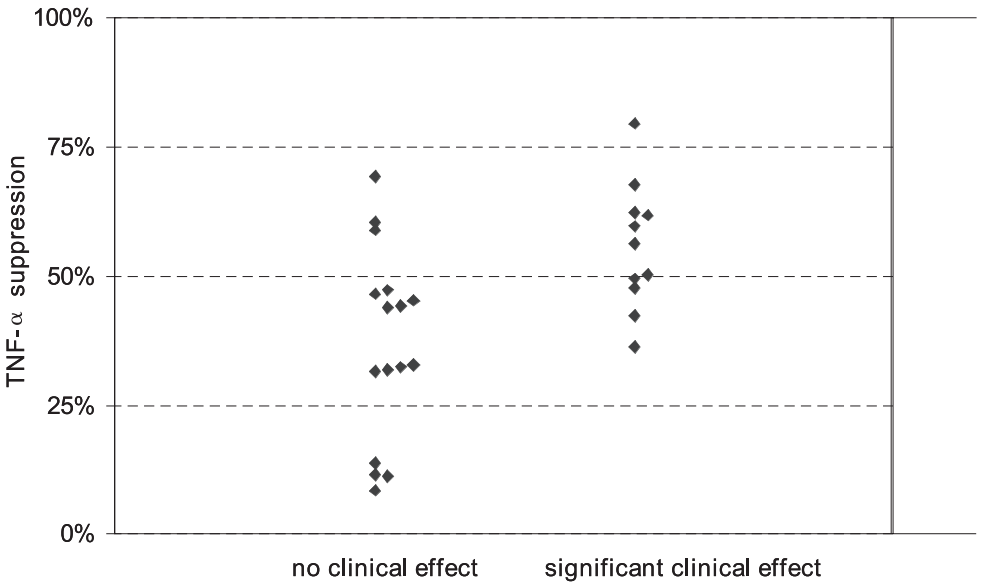

Figure. Relation between the clinical effect of IVMP treatment and the *in vitro* suppression of TNF-α production by dexamethasone (10^{-6}M, DEX-6) in PBC collected prior to therapy. The difference between the groups is statistical significant (Mann-Whitney U test, p=0.007).

In turn, the majority (7/10) of patients with 50% or more suppression of the TNF-α production in the presence of DEX-6 showed a significant clinical improvement in response to IVMP treatment as opposed to a minority (4/17) of the patients with a suppression of less than 50%, without improvement. This leads to a relative risk of 3 (Fisher's exact test, p=0.040).

Discussion

We hypothesized that the beneficial effects of MP treatment are, at least in part, mediated by its suppressive effect on pro-inflammatory cytokine production, and that this can be used as a measure for GC sensitivity. Our findings show that PBC of the group without a clinical effect exhibit a lower responsiveness to steroids *in vivo* as well as *in vitro* as compared to those that experience a clinical effect.

Persistent production of pro-inflammatory cytokines by immunocompetent cells even in the presence of considerable concentrations of GC probably accounts for an ongoing immune reaction. This partial GC resistance may contribute to the lack of clinical response to IVMP. In case this resistance also holds for endogenous GC, this may result in an inability to properly down regulate the inflammatory response and thus a more active disease. Such an active disease might urge the need for GC treatment but, as a consequence of this partial GC resistance, the effect of this treatment will probably be disappointing.

If confirmed in future studies the *in vitro* effect of steroids on PBC might be used to predict

the chance of significant clinical improvement in response to IVMP treatment in MS. This could contribute to a more individually tailored approach in MS.

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