Glucocorticoid sensitivity in multiple sclerosis; what makes the difference?

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Summary

Glucocorticoids (GC) are hormones produced by the adrenals under influence of various stress factors. Their production is controlled by the hypothalamus-pituitary-adrenal (HPA) axis. GCs exert their function via glucocorticoid receptors (GRs), which are present on nearly every cell in the body. GCs influence a broad array of functions in the body, among others, they have anti inflammatory effects. It has been suggested that GCs act as a mediator between the nervous system and the immune system. Furthermore previous studies have shown that in healthy persons as well in patients with various chronic inflammatory diseases, differences in GC sensitivity appear.

This thesis evaluates different aspects of glucocorticoid (GC) sensitivity in patients with multiple sclerosis (MS). To be more specific differences in peripheral GC sensitivity and polymorphisms in the glucocorticoid receptor (GR) gene leading to differences in GC sensitivity will be adressed.

Exogenous GCs are widely used for the treatment of chronic inflammatory diseases, such as multiple sclerosis (MS), because of their anti-inflammatory properties. In the second part of this thesis we evaluate various outcome measurements in their ability to detect changes after intravenous methylprednisolone (IVMP) therapy which is an exogene GC.

In **chapter 1**, general information on MS is given. Epidemiologic and genetic factors, diagnosis, clinical course and treatment options are adressed. Exogene and endogene GCs and the regulation by the hypothalamic-pituitary aderenal (HPA) axis are discussed in more detail. Also the mechanisms through which GCs exert their effects via binding to the GR are described. This is to understand various factors that possibly influence GC sensitivity. We focussed on variability in the GR gene as one of the factors influencing GC sensitivity.

Chapter 2 Glucocorticoid sensitivity in MS

There is strong evidence for HPA-axis hyperactivity in MS-patients, which goes along eith increased cortisol levels. Despite elevated cortisol levels, clinical signs of hypercortisolism in MS are unusual. Taken together, these findings suggest that increased HPA axis activity is accompanied by a reduction in the peripheral GC sensitivity in MS patients. In **chapter 2.1**, we evaluated this hypothesis. GC sensitivity was measured by the in vitro suppressive effect of GC on lipopolysaccharide (LPS) stimulated TNF-α production in a whole blood assay in 117 MS patients and 45 controls. Blood cells of MS patients, especially relapsing remitting (RR) MS patients, turned out to be less sensitive to GC compared to controls. This difference in sensitivity was not related to previous treatment with exogenous GC. This was expressed as the frequency of courses of IVMP as well as interval since the last course. The use of interferon β (IFNβ) was found to be associated with a lower GC sensitivity. However, also after controlling for the use of IFNβ, RRMS patients remained less sensitivitive. IFNβ can influence the HPA axis activity, resulting in increased endogenous cortisol levels. In our study, IFNβ treatment was associated with lower GC sensitivity. IFNβ treatment is especially initiated in patients

with a clinically active disease course. This association is probably caused by an increased and ongoing inflammation in the CNS. This increased and ongoing inflammation may in turn be related to or – via a defective suppression by endogenous GC – even caused by a decreased GC sensitivity. We suggest that the observed effect of IFNβ should perhaps not be attributed to IFNβ itself but rather to selection bias in the sample. Indeed, after careful comparison of the disease characteristics of our RR and SP patients we observed a significant difference in exposure to IVMP treatment thereby supporting the latter explanation.

Various mechanisms may underlie differences in GC sensitivity. One mechanism could be genetically variation in the gene of the GR. In **chapter 2.2** we tested the hypothesis that carriers of single nucleotide polymorphisms (SNP) of the GR gene (N363S, ER22/23EK, and the B*cl* I), which are associated with a decreased or increased GC sensitivity, have decreased respectively increased sensitivity of peripheral blood-cells *in vitro* in MS patients and controls. In controls we found an association between the N363S allele of the GR-gene and a reduced peripheral GC sensitivity *in vitro*. In MS patients neither the N363S, B*cl* I, nor the ER22/23EK allele were found to be associated with GC sensitivity. Although this study was based on a small sample, this suggests that GC sensitivity is probably in part genetically influenced in healthy controls, but in MS patients also other factors play a role in GC sensitivity in MS patients. For example cytokines, IFNβ, or other unknown factors which seem to have more influence than the genetic effect on GC sensitivity.

Chapter 3 Clinical implications of differences in the GC sensitivity

GC sensitivity and clinical effect after IVMP

GCs are widely used for the treatment of chronic inflammatory diseases. In several inflammatory or autoimmune diseases including rheumatoid artitis (RA), asthma, M. Crohn, and ulcerative colitis, subgroups of patients who are clinically less responsive to GC have been distinguished. This has been associated with decreased GC sensitivity of blood cells. In chapter 3.1 we hypothesized that MS patients with who respond clinically (i.e. had e reduced disability level) to IVMP are sensitive to GCs and vice versa. To test this proposition, 27 MS patients, who were treated with IVMP, because of a relapse, were studied prospectively. GCs can inhibit the production of pro-inflammatory cytokines like tumor necrosis factor α (TNF-α). This supressive effect of GCs on LPS stimulated TNF-α production was used to determine GC sensitivity. Before and after treatment *in vitro* LPS stimulated TNF-α production in blood cells and the effect of in vitro administered GC were measured. We evaluated whether *in vitro* as well as *in vivo* GC sensitivity before and after treatment with IVMP, was related to the clinical effect. Furthermore we analysed whether *in vitro* GC sensitivity before treatment could predict the clinical effect. We found that 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. These results suggest the existence of a partial GC resistance. 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. Consequently, this could lead to 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.

Glucocorticoid receptor gene variation and disease course

Endogenous GCs are considered to restrain the immune system in such a way that the probability of recovery from relapse is increased. In MS patients, lifelong decreased GC sensitivity, due to single nucleotide polymorphisms (SNPs) of the GR gene, may result in ongoing inflammation which in turn may influence the clinical course. We investigated three SNPs (N363S, ER22/23EK and B*cl* I C/G) of the GR gene, which have been previously associated with altered GC sensitivity *in vivo*, for their potential role in disease course and susceptibility. In earlier studies, the ER22/23EK is associated with higher cortisol levels after dexamethasone suppression tests (DST), indicating that carriers of this allele are relatively more resistant to the effects of GCs. Indeed, we found that the ER22/23EK polymorphism, was associated with a more aggressive MS phenotype, measured both clinically and on MRI. Even though MRI data were only available in a subgroup of patients, carriers of the polymorphism ER22/23EK, had a larger T1 weighted lesion volume compared to non carriers. This finding could be considerd as a correlate of axonal loss. These findings suggest that for patients having the the ER22/23EK polymorphism both augmented inflammatory activity and more extensive neuronal degeneration may account for the increase in disability progression in MS (**chapter 3.2**).

In **chapter 3.3** we replicated previous studies in a larger population. We took into account the effect of other SNPs (9β-G and TthIIII, besides the previous studied Bcl I C/G, ER22/23EK and N363S) by inferring haplotypes. A haplotype is a set of SNPs on a single chromatid that are statistically associated. It is thought that these associations, and the identification of a few alleles of a haplotype block, are representative for other polymorphic sites in its region. In 646 MS patients and 317 HCs we investigated whether haplotypes including the ER22/23EK polymorphism or the GR 9β polymorphism, which is also associated with a relative GC resistance, were associated with a more aggressive disease course. The association with disease progression was analyzed using Cox regression with time to Expended Disability Status Score 6 as outcome.

None of the haplotypes was associated with disease susceptibility, age at onset or onset type. Haplotype 6 (Tth*III*I, ER22/23EK, 9β-G), was associated with a more rapid disease progression. This seems to result from the presence of ER22/23EK and not from the 9β and Tth*III*I SNPs.

The disease course of MS patients carrying the haplotype 6 (Tth*III*I, ER22/23EK, 9β), is more aggressive. This is probably due to the presence of the SNP ER22/23EK. As such, the effects of these polymorphisms might be small. However, the combined effects of life long exposure and those resulting from the complex feed back circuits between the HPA axis and the immune system, render the impact of these polymorphisms on disease course plausible.

Chapter 4 Measurement of treatment effect after IVMP

In MS, the measurement of responsiveness to treatment is complicated . This is so because of the large variation of neurological symptoms. Furthermore, symptoms like fatigue and cognitive problems lack a precise clinical definition. This makes it difficult to find accurate outcome measures to distinguish which patients do clinically respond to IVMP, and who do not. Since long the Expanded Disability Status Score (EDSS) has been used to evaluate treatment effect in MS, or disease progression. Because the development of new treatments in the last two decades, as well as a growing awareness of the importance of patient-oriented measures, new outcome measures have been developed which aim to be more responsive to changes and clinically relevant to patients as well. In **chapter 4.1** we compared a physician-oriented (MS functional composite, (MSFC)) and patient-oriented (Guys neurological disability scale (GNDS)) clinical outcome measure with the EDSS concerning the ability to detect improvement after IVMP treatment. Sixty MS patients were treated with IVMP. On the first day of treatment patients were trained for the three domains of the MSFC: Timed 25-foot Walk (T25FW) assessing leg/ambulation, 9-Hole Peg (9-HPT) to assess the arm/hand function and cognition was tested by the 3" version of the paced auditory serial addition test (PASAT). On the second day baseline data were obtained for all measurements (MSFC,EDSS and GNDS). Follow-up data were obtained 6-8 weeks after IVMP treatment. Significant changes were found for both EDSS and GNDS. Twenty one patients showed a significant improvement in the EDSS, 28 patients showed a significant improvement in the GNDS. Remarkably, the improvements on the GNDS were mainly due to changes in the subcategories cognition, speech, fatigue and 'others' and less due to the effects on other categories. Forty-seven patients reported a subjective improvement in their condition. These findings show that the relative sensitivity to change is low for the MSFC. Even though we identified a number of variables that might affect the sensitivity to change for the respective scales (e.g. the importance of the selection of a good reference population for the MSFC, and the three point cut off point for change of the GNDS), we suggest that further studies should be performed to study their behaviour under different conditions.

The suggestion that corticosteroids might especially have an effect on subjective features of well being is certainly supported by the data collected in this study. It is obvious from our study that a treatment can have a differential effect on measurements of functional impairment, rating of neurologic exam and patient self-report.

The ability of different outcome measures detecting improvement is sparsely studied. In **chapter 4.2** we evaluated the predictive accuracy to distinguish a clinically meaningful change (from the viewpoint of the patient) of two quantitative tests (T25FW and the 9-HPT) and the EDSS. This study was based on a sample of 101 MS patients before and 6 weeks after IVMP. The influence of baseline disability, based on baseline EDSS score at responsiveness was studied. In addition patients were asked to rate their change as an anchor to assess the performance of the tests.

Putting the T25FW and the 9-HPT together turned out to be the optimal combination

of measures to predict patient's perceived change. In the higher EDSS range (EDSS 4.5 and higher) for all measures a significant change was more often perceived as clinically relevant than in the lower disability range.

The results from this study, although based on a relatively small sample, suggest that the EDSS is not the preferred method to detect patient perceived improvement in MS, especially in the lower EDSS range. Combining T25FW and 9-HPT can improve the sensitivity to detect clinically relevant changes without conceding with respect to specificity of this test. The use of combinations of outcome measures in MS should be further explored about ther ability te detect clinically meaningful changes.

Chapter 5 Discussion and future perspectives

In **chapter 5** the results of this thesis are summarized and discussed, and placed in the light of the current knowledge about the pathogenesis of MS. Also, future perspectives are presented. As a final conclusion, blood cells of MS patients have a decreased GC sensitivity. We hypothesize that in general GC sensitivity is a at least partly genetically determined factor, which is additionally influenced by more fluctuating factors such as inflammation, IFNβ treatment and other, yet unknown, factors.

The SNP ER22/23EK decreases GC sensitivity and has been associated with increased HPA axis activity, which is thought to reflect a disturbance in the negative feedback of the HPA axis. This combination results in an unfavourable situation in patients with chronic inflammation, like in MS. We found that the haplotype TthIII1, ER22/23EK, 9β, is associated with a more aggressive disease course in MS. Further studies are warranted to investigate whether this haplotype can be used as prognostic factor among other factors, to help to identify patients who will develop an aggressive disease course.

Second, in this thesis we evaluated outcome scales to measure treatment responses to IVMP. We found additional evidence that the EDSS seems less responsive to changes that are clinical meaningful from the patients' perspective. We showed that combined measures, like the T25FW and the -HPT, allows us to distinguish a higher number of patients who show a clinically significant change, without leading to a concomitant loss of accuracy in terms of being relevant to the patient.

This may help to define more optimal outcome measures since both outcome scales that assess improvement after IVMP, as well as outcome scales that evaluate disease course (e.g. in trials), have to be clinical relevant for the patients.