Dexamethasone in the primary prevention of rheumatoid arthritis: a randomized trial

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Abstract

Objective. Several autoimmune diseases are characterized by autoantibodies in the preclinical phase. Therefore, an intervention aimed at decreasing autoantibody levels may be effective in delaying or preventing progression to overt disease. In rheumatoid arthritis the relevant autoantibodies are antibodies to citrullinated proteins (ACPA) and IgM rheumatoid factor (IgM-RF).

Methods. In a double-blind trial, 83 arthralgia patients positive for autoantibodies (ACPA or IgM-RF) and positive for the major genetic risk factor HLA-DRB1 ‘shared epitope’ were stratified for ACPA status and then randomly allocated to intramuscular injections of 100 mg dexamethasone or placebo at t=0 and 6 weeks. Before randomization two investigators independently confirmed the absence of arthritis. The primary endpoint was the antibody reduction at 6 months.

Results. Patients treated with dexamethasone but not placebo showed specific and substantial reductions of antibody levels, with a nadir after 1 month. At that point, median change in ACPA levels was -22% (IQR -29 : -14%) in the dexamethasone group versus +3% (IQR -13 : +6%) in the placebo group (P<0·001). For IgM-RF, corresponding results were -14% (IQR -1 : -28%) versus -1% (IQR -9 : +17%; P=0·003). The reduction persisted up to 6 months for ACPA. During a median follow-up of 26 months (range 12-51), a similar proportion of patients developed arthritis in both groups (20% vs. 22%; median 4 joints).

Conclusion. In autoantibody positive arthralgia patients, treatment comprising two injections of 100 mg of dexamethasone substantially decreases ACPA and IgM-RF levels, but does not delay or prevent arthritis development after 26 months.

Trial nr: ISRCTN73232918 (www.trialregister.nl)

Introduction

Several autoimmune diseases such as systemic lupus erythematosus, type I diabetes mellitus and rheumatoid arthritis (RA) are characterized by autoantibodies in the preclinical phase, thereby enabling the detection of individuals at risk. Antibodies to citrullinated proteins (ACPA) and IgM rheumatoid factor (IgM-RF) in RA are a well-described example, and ACPA are highly sensitive and specific for RA in the presence of arthritis. We have reported the presence of ACPA and/or IgM-RF in 49% of blood donors who later developed RA; seroconversion occurred at a median of 5 years before the onset of symptoms. The risk of the development of RA within 5 years in the presence of these antibodies was estimated at 2% in the general population, increasing to 44% in persons having multiple family members with RA. It can be assumed that the presence of arthralgia or of the major genetic risk factor for RA, the shared epitope (SE) at the HLA-DRB1 locus, may also increase the risk for RA development.

RA is a systemic autoimmune disease, characterized by a chronic destructive joint inflammation, reduced functional capacity and increased mortality. A severe disease course is predicted by IgM-RF and even more so by ACPA. Possible pathophysiological properties of ACPA, and to a lesser
extent IgM-RF, are suggested by the presence in the preclinical or asymptomatic phase of the disease,\textsuperscript{2;3;16-19} the association with a more severe disease course and the ability of ACPA to induce or enhance arthritis in rodents.\textsuperscript{20;21}

Therefore, an intervention aimed at decreasing autoantibody levels in persons with such antibodies may delay or prevent the development of RA. In RA patients glucocorticoids, disease modifying antirheumatic drugs and biologicals are all capable of reducing autoantibody levels, although the extent of these effects varies considerably between drugs and between studies.\textsuperscript{22-27} High-dose glucocorticoid treatment reduces IgM-RF levels in RA patients by more than 50\%\textsuperscript{22-24} A recent study showed a 50\% ACPA reduction after 8 weeks in early arthritis patients using a combination of glucocorticoids and disease modifying antirheumatic drugs (the COBRA scheme\textsuperscript{28}), suggesting a possible effect of glucocorticoids on ACPA (van Tuyl et. al., submitted).

Early treatment of RA seems to result in a greater than usual response to therapy with sustained benefit, the ‘window of opportunity’.\textsuperscript{29} However, about one third of patients with early (rheumatoid) arthritis already have radiographic erosions at the first clinic visit, shortly after the onset of symptoms.\textsuperscript{30} Therefore, to prevent the disease burden associated with RA the logical next step is to target persons at risk for the development of RA, i.e. before the onset of arthritis.

In the present study we report the results of a randomized double blind placebo-controlled trial of dexamethasone in ACPA and/or IgM-RF positive arthralgia patients aimed at the reduction of autoantibody levels and the prevention of arthritis development.

* Two patients in each group were lost to follow-up after inclusion and baseline values were carried forward.
Patients and methods

Study Participants. Between June 2004 and 2007, ACPA and/or IgM-RF positive arthralgia patients were recruited at rheumatology clinics in the Amsterdam area of the Netherlands. After screening and global informed consent by telephone, autoantibodies and SE genotyping were determined at least one month after the first positive sample; one or both antibodies had to be above the threshold for test positivity at both occasions to allow for inclusion. SE positivity was an additional entry criterion, because of supposed risk enhancement in these patients. At the first study visit, a trained medical doctor (WB) and a senior rheumatologist (DS) independently scored for absence of arthritis in 44 joints at physical examination. The senior rheumatologist was blinded for the reported joint complaints and the autoantibody status. Disagreement resulted in exclusion from the study. Of 227 patients thus recruited, 38 were excluded by the following criteria: arthritis revealed by chart review (n=5) or baseline physical examination (n=31), previous treatment with a disease modifying anti-rheumatic drug (DMARD; n=2).

The remaining exclusion criteria were not seen in any patient: erosions on hand or feet X-ray examination, recent (< 1 year) infection or malignancy (because of possibility of false positive IgM-RF), other autoimmune diseases possibly associated with positive IgM-RF (Sjögren’s disease or SLE), recent (< 3 months) glucocorticoid treatment, diabetes mellitus, osteoporosis and pregnancy or lactation (because of the risk of glucocorticoid-induced side effects).

Furthermore, 90 patients were negative for the SE and 16 patients refused dexamethasone treatment; therefore 83 antibody (ACPA and/or IgM-RF) positive arthralgia patients were eligible for randomisation. Figure 1 shows the flow chart of the inclusion process.

Study Protocol. Details of joint complaints were recorded at baseline, after 3, 6 and 12 months and during yearly follow-up visits. Extra visits were planned if the patient developed arthritis. At such visits, soft tissue swelling in any of the 44 joints was independently confirmed by the same two investigators who performed the inclusion visit, and the disease activity score of 28 joints (DAS28) was determined as well as whether the patient fulfilled the ACR criteria for RA.

The intervention comprised an intramuscular injection of either 100 mg of dexamethasone or placebo. Identical appearing verum and placebo vials were prepared by the pharmacy of the VU University Medical Center. Randomisation was performed by the pharmacy by sequential allocation to prefixed numbered study medication, with stratification by ACPA status in blocks of four in a 1:1 ratio. Patients who entered the study were assigned a unique study identification number corresponding to the vial containing the study medication. The assignment was only known to the personnel of the pharmacy. The injection was given by a trained nurse and was repeated once after six weeks. To prevent excess treatment, only those dexamethasone treated patients who had not reached a 50% reduction of one or both antibodies after 4 weeks were given a second dexamethasone injection. The placebo group and the patients reaching a 50% reduction at 4 weeks were given a placebo injection at 6 weeks to prevent deblinding of the trial. The antibody results at 4 weeks were only revealed to the pharmacist, who then instructed the investigator to use the appropriate coded vial for the second injection at 6 weeks. Safety
was assessed with adverse event reports recorded six weeks after each injection. The study was approved by the local ethics committee and all patients gave written informed consent.

**Laboratory investigations.** ACPA and IgM-RF levels were determined batchwise at the end of the study period using the serum samples that were obtained at inclusion and 1, 3, 6 and 12 months after the first injection. Assays comprised a second-generation anti-CCP enzyme-linked immunosorbent assay (ELISA; Axis Shield, Dundee, United Kingdom) and an in-house ELISA as described previously, respectively. The cut-off level for anti-CCP antibody positivity was set at 5 arbitrary units/ml (AU/ml) according to the manufacturer's instructions. IgM-RF was calibrated with a national reference serum containing 200 international units (IU/ml), the cut-off level for IgM-RF antibody positivity was set at 30 IU/ml determined on the basis of ROC curves as described previously. Total IgM and IgG levels were measured at all time points using Tina-quant Gen.2 reagents with module C501 of a COBAS 6000 platform (Roche Diagnostics GmbH; Mannheim, Germany). High sensitivity c-reactive protein (CRP) levels (normal range < 10 mg/L) were determined using the Roche/Hitachi cobas c systems with a detection range of 0.15-20 mg/L. Samples reaching 20 mg/l were diluted further. The test principle consists of a particle enhanced immuno-turbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies.

The SE was deduced from HLA-DQ typing as described previously. Since this is an indirect genotyping method with possible false positivity in non-Caucasians, HLA-DRB1 genotyping was performed by sequence based high resolution typing (Sanquin, Amsterdam, the Netherlands) at the end of the study. The HLA-DRB1 *0101, *0102, *0401, *0404, *0405, *0408, *0410 and *1001 alleles were taken to contain the SE. The latter method showed that 75/83 = 90% had been correctly classified as containing the SE sequence by the DQ method.

**Analysis.** A sample size of 80 (40 per group) was precalculated to obtain 80% power with a 5% significance level to detect a difference between the presence or absence of ≥ 50% reduction level or normalization of ACPA or IgM-RF at 1, 3 and 6 months, which was arbitrarily chosen due to the lack of relevant data. Data obtained from the previously described blood donor population were used as untreated controls in the power calculation. Outcomes were calculated in an intention-to-treat (ITT) analysis using all available data. The primary analysis was done with coded group allocation after entry of all study data. The randomisation codes remained concealed until completion of the primary outcome measure analysis.

The primary outcome measure, arbitrarily defined as a 50% reduction of one or both autoantibody levels at 6 months, was analysed using Fisher’s exact test. Between group comparisons of antibody levels at each time point were assessed using Mann-Whitney U test. Analysis was done with antibody positive patients only and for both antibodies separately. Cox-regression hazard analysis assessed the relative risk for arthritis development in the placebo group compared to the dexamethasone group.

Two patients in each treatment group were lost to follow-up after inclusion; baseline values of antibodies were carried forward. Exclusion of these patients did not alter the results. The analyses were performed using SPSS version 15.0 (Chicago, Illinois).
Role of the funding source. The funding source did not have any involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Results

Baseline characteristics. The randomisation of 83 arthralgia patients was successful in creating balanced groups (table 1). In particular, stratification for ACPA led to similar median ACPA and IgM-RF levels. The median onset of symptoms was at one year before the baseline visit. Two patients in each group were lost to follow-up after inclusion and baseline values were carried forward.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>dexamethasone n=42</th>
<th>placebo n=41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD) years</td>
<td>45 (10)</td>
<td>48 (10)</td>
</tr>
<tr>
<td>Female sex</td>
<td>26 (62)</td>
<td>27 (66)</td>
</tr>
<tr>
<td>Symptom duration in months, median (IQR)</td>
<td>12 (6-36)</td>
<td>12 (6-24)</td>
</tr>
<tr>
<td>Number of tender joints, median (IQR)</td>
<td>8 (2-12)</td>
<td>4 (1-14)</td>
</tr>
<tr>
<td>Distribution of tender joints‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small joints</td>
<td>9 (21)</td>
<td>5 (12)</td>
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<tr>
<td>Large joints</td>
<td>8 (19)</td>
<td>15 (37)</td>
</tr>
<tr>
<td>Small and large joints</td>
<td>25 (60)</td>
<td>21 (51)</td>
</tr>
<tr>
<td>Symmetric distribution of tender joints‡</td>
<td>34 (81)</td>
<td>25 (61)</td>
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<tr>
<td>Localisation of tender joints‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper extremities</td>
<td>20 (48)</td>
<td>20 (42)</td>
</tr>
<tr>
<td>Lower extremities</td>
<td>2 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Upper and lower extremities</td>
<td>20 (48)</td>
<td>19 (46)</td>
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<tr>
<td>Morning stiffness for more than one hour</td>
<td>8 (19)</td>
<td>3 (7)</td>
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<td>Pain on a 100 mm VAS, median (IQR)</td>
<td>25 (0-49)</td>
<td>20 (0-48)</td>
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<tr>
<td>Number of tender joints at physical examination, median (IQR)</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
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Autoantibody status

<table>
<thead>
<tr>
<th>Autoantibody status</th>
<th>dexamethasone n=42</th>
<th>placebo n=41</th>
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</thead>
<tbody>
<tr>
<td>IgM-RF positive, ACPA negative</td>
<td>10 (24)</td>
<td>12 (29)</td>
</tr>
<tr>
<td>ACPA positive, IgM-RF negative</td>
<td>18 (43)</td>
<td>13 (32)</td>
</tr>
<tr>
<td>IgM-RF and ACPA positive</td>
<td>14 (33)</td>
<td>16 (39)</td>
</tr>
<tr>
<td>ACPA (AU/ml), median (IQR)§</td>
<td>63 (14-244)</td>
<td>71 (21-201)</td>
</tr>
<tr>
<td>IgM-RF(IU/ml), median (IQR)§</td>
<td>82 (52-125)</td>
<td>58 (39-103)</td>
</tr>
</tbody>
</table>

*Except where indicated otherwise, values are the number of patients (%). SD = standard deviation, IgM-RF = rheumatoid factor, ACPA = anti-citrullinated protein antibodies, IQR = interquartile range, VAS = visual analogue scale.

‡ n=74.

‡ during current symptom period.

§ positive patients only.

Autoantibody levels. For ACPA, no patient reached the primary target of ≥ 50% reduction in antibody levels, and for IgM-RF one patient in each group reached that target. Nevertheless, only the dexamethasone group showed significant and substantial reductions of the levels of both antibodies, with a nadir after 1 month (Figure 2). At that point, median (IQR) change in ACPA levels was -22% (IQR -29 : -14%) in the dexamethasone group vs +3% (IQR -6 : +13%) in the placebo group (P <
For IgM-RF, corresponding results were -14% (IQR -28 : -1%) vs +1% (IQR -9 : +17%; \( P = 0.001 \)). Reductions persisted up to month 6, although the difference between the groups was no longer significant for IgM-RF at that time (-13% [IQR -27 : 0%] and -7% [IQR -24 : +2%] for ACPA, -7% [IQR -26 : +15%] and -2% [IQR -9 : +11%] for IgM-RF in the dexamethasone group at 3 and 6 months, respectively, figure 2). The individual attained percentages of pre-treatment values, plotted as cumulative probability plots is shown in figure 3. ACPA and IgM-RF levels decreased significantly in time in the dexamethasone group (\( P < 0.001 \) and \( P = 0.01 \), respectively), but not the placebo group (\( P = 0.52 \) and \( P =0.56 \), respectively).

An additional analysis at 12 months showed that the difference in ACPA change between the groups persisted (-23% [IQR -36 : +3%] versus -8% [IQR -16 : +12%], respectively; \( P = 0.03 \) for between-group comparison). For IgM-RF, this effect at 12 months was not observed. However, whereas disease modifying antirheumatic treatment was absent in the first 6 months, treatment with such drugs of those who developed arthritis later on might have influenced these results.

Qualitative changes were not observed; none of the patients positive at baseline turned negative during follow-up; the reverse was also not observed; autoantibody negative patients remained negative.

**Total immunoglobulin levels.** Total IgG and IgM levels were determined to investigate whether the observed decrease in autoantibodies was specific. A small change in lowest reached total IgG and IgM value was observed, which was similar in both treatment groups (median values -12% versus -5% for IgG and -7% versus -6% for IgM in the dexamethasone and placebo group, respectively). In the dexamethasone group, the change in IgG ACPA (-25%) was greater than for total IgG levels (-12%; \( P < 0.001 \)) and the change in IgM-RF (-19%) was greater than for total IgM levels (-7%; \( P = 0.004 \)). Therefore, the small non-significant decrease in autoantibodies in the placebo group probably reflects changes in total immunoglobulin levels, whereas the effect of dexamethasone on autoantibodies is not a reflection of the decrease in total immunoglobulin levels.
Arthritis development. After a median follow up of 26 (IQR 21-37) months in both groups, 9/42 patients in the intervention group and 8/41 patients in the placebo group had developed arthritis in a median of 4 (IQR 3-7) joints; 6/17 fulfilled the 1987 American College of Rheumatology criteria for RA (3 in both treatment groups). Median DAS28 score was 2.9 (IQR 2.4-4.2) in the dexamethasone group and 3.7 (3.2-4.1) in the placebo group. The between-group hazard ratio (HR) for arthritis development was 1.1 (95% CI 0.4-2.8; P = 0.9; figure 4). At 6 months, 3 patients (2 in the placebo group) showed an increase in ACPA levels of more than 80% compared to the baseline value (figure 3). All 3 developed arthritis (after 1 month and 4 months in the placebo group and after 15 months in the dexamethasone group).
Safety. The injections were generally well tolerated. Serious adverse events did not occur. Sixteen patients (20%) reported mild adverse events after the first injection, which all resolved spontaneously within 3 days. Twelve comprised tenderness at the injection site resolving within one day. This recurred in a milder fashion in 3 patients. Adverse events were distributed equally over both treatment groups.

Discussion

In this first trial of primary prevention in persons at risk of developing RA, intramuscular dexamethasone induced substantial, specific and sustained decreases in ACPA and IgM-RF levels. This result was not paralleled by a delay or decrease of the development of arthritis after 26 months.

The ACPA response to dexamethasone was greater than that of IgM-RF. Taking the half-lives of IgG and IgM into account (23 and 5 days, respectively), the differential effect is even greater. Therefore, ACPA may be a more responsive target than IgM-RF in the preclinical phase, in contrast to established RA, where IgM-RF appears to be more responsive: IgM-RF may decrease by 50% and ACPA by 25% during antirheumatic treatment of RA.\textsuperscript{22-25,27,36,37} These results suggest properties as disease activity marker for IgM-RF and disease specificity marker for ACPA.\textsuperscript{38} The apparent differential effect on IgM-RF and ACPA is probably not attributable to a difference in clearance rate between the IgG subclass of ACPA and the IgM subclass of IgM-RF, since the reduction of IgM-RF and IgG-RF isotypes after anti-tumor necrosis factor alpha or rituximab treatment is equal.\textsuperscript{25,27}
The selective, substantial and sustained reduction of ACPA and IgM-RF levels beyond their molecular half-lives is in line with observations in established RA.\textsuperscript{23,39} It suggests that the B cell precursors of autoantibody producing plasma cells are sensitive to the lytic properties of dexamethasone, and therefore that the levels of ACPA and IgM-RF are dependent on the constant generation of new plasma cells from B lymphocytes, driven by antigenic stimuli such as e.g. citrullinated proteins.\textsuperscript{40}

Previous preventive efforts in autoimmune diseases have concentrated on type I diabetes. Trials of enteral or parenteral insulin and of nicotinamide in relatives of patients with diabetes were not successful in delaying or preventing diabetes.\textsuperscript{41-43} In contrast to these studies, we applied direct immune-suppression in the preclinical phase of RA. Despite a reduction in ACPA and IgM-RF levels, that at least for ACPA was similar to what was achieved with the most effective therapies in established RA, two injections of 100 mg of dexamethasone were not sufficient to delay or prevent the development of arthritis, which was similar in both groups. The figure of 20\% arthritis development within two and a half years shows that the risk of arthritis development in autoantibody positive arthralgia patients is considerable. We have previously estimated the risk for arthritis development at 2 - 44\% within 5 years in a population of autoantibody positive blood donors without joint complaints.\textsuperscript{3} It is therefore likely that the arthralgia of autoantibody positive persons is often caused by the rheumatoid inflammatory process that has not yet reached sufficient intensity to be detectable clinically.

In the present cohort, the number of new cases with arthritis will most probably increase with prolonged follow-up, however, a doubling of arthritis cases in the placebo group without new cases in the dexamethasone group would be needed to reach a statistically significant difference between both treatment arms. Although dexamethasone does not seem to have an effect on arthritis development, it may still lead to a milder arthritis course, as such an effect was suggested by glucocorticoid treatment of early oligoarthritis, although a control group was lacking.\textsuperscript{44} Follow-up of the patients who have developed arthritis will have to reveal this. This effect is suggested by the lower disease activity score at arthritis onset in the dexamethasone group when compared to the placebo group, although the present study was not designed to detect such differences.

The question arises whether the present intervention was the most appropriate. A longer course of glucocorticoids and/or other disease modifying antirheumatic drugs or even biologicals might have been more successful in reducing arthritis development. However, a longer or more intensive regimen than the one employed could have met with considerable ethical and compliance issues. Future studies attempting primary prevention of RA can either focus on a more effective regimen of immune-suppression or employ a tolerogenic strategy, e.g. directed against citrullinated proteins. The latter approach already has proven effective in mice.\textsuperscript{20} However, despite successes in rodents, tolerogenic strategies in the treatment of human auto-immunity, including enteral collagen treatment and nasal treatment with human cartilage glycoprotein 39 in RA, have thus far not been very successful.\textsuperscript{45-47}

One can argue that patients presenting with autoantibody positive arthralgia already represent a very early stage of RA. However, the absence of arthritis confirmed by two experienced investigators, a median of zero tender joints at physical examination and CRP levels in the normal range make the
presence of clinically detectable synovitis is unlikely. Imaging techniques such as ultrasound may reveal subclinical synovitis in these patients, this is a subject of ongoing investigation.

In conclusion, two intramuscular dexamethasone injections in autoantibody positive arthralgia patients substantially decreases ACPA and to a lesser extent IgM-RF levels, but does not delay or prevent arthritis development. Ongoing long term follow-up will further clarify the effects of this very early intervention strategy.

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Conflict of interest statement. DS had full access to all the data in the study and had final responsibility for the decision to submit for publication. BD, MB, RS and DS have participated in the study design; WB, RS and DS in the acquisition of the data; WB, BD, MB and DS in the data analysis and interpretation of the data. All authors have participated in the preparation of the manuscript, have seen and approved the final version and have no conflicts of interest.
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Primairy prevention of rheumatoid arthritis


