Treatment of juvenile idiopathic arthritis with intra-articular triamcinolone hexacetonide: Evaluation of clinical effectiveness correlated with circulating ANA and T γ/δ + and B CD5 + lymphocyte populations of synovial fluid

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ABSTRACT

Objective

The aims of the study were to assess the effect of intra-articular treatment with triamcinolone hexacetonide (TH) in juvenile idiopathic arthritis (JIA) and to investigate whether treatment response correlates with the presence of antinuclear antibodies (ANA) in the serum and/or B CD5+ and T γ/δ+ lymphocytes in the synovial fluid.

Methods

A total of 37 patients (81% females, 56% ANA+) with oligoarticular JIA involving knees were treated with intra-articular injections of TH after failing to respond to NSAIDs for two months. Eighteen patients were treated within 6 months of onset, 19 were treated more than 6 months after onset.

Result

Mean duration of remission was 13.9 months. Twelve patients (7 ANA+) had stable remission after a single injection; 13 patients (3 ANA+) experienced more than 6 months' remission but subsequently had a relapse; 12 patients (11 ANA+) had a relapse within six months of injection. Of 20 patients treated within 6 months of onset, 17 had stable remission whereas only 8 out of 17 who were treated during relapse attained stable remission (p = 0.03). The mean percentage of T γ/δ+ and of B CD5+ lymphocytes in synovial fluid was the same as in peripheral blood of normal subjects.

Conclusion

Our data indicate that local treatment with slow-release steroids is very effective in oligoarticular JIA. Prolonged remission was less likely in the presence of ANA positivity, probably because the disease is immunologically more active. Finally, our data suggest that the earlier the treatment, the easier it is to obtain a protracted, and possibly permanent, response.

Introduction

The therapeutic approach to juvenile idiopathic arthritis (JIA) oligoarticular form has been revised by local hydrocortisone-based treatment with long-acting properties, such as triamcinolone hexacetonide (TH), a glucocorticoid ester in a crystal preparation which is less soluble, less rapidly hydrolyzed and therefore has a more lasting effect. Only local side effects have been reported for this treatment (1, 2) and a number of studies have reported encouraging results, with stable remission (at two years' follow-up) after a single injection in more than 60% of patients. (3)

However, there have been few studies in patients with JIA and even fewer into the correlation between clinical response and laboratory immunologic parameters. The aim of this prospective study is to assess the effect of intra-articular treatment with triamcinolone hexacetonide injection into the knee in a group of patients with JIA.

We also investigated the correlation between clinical response and presence of ANA in peripheral blood, as well as studying the T γ/δ+ and B CD5+ lymphocyte subsets in synovial fluid, since it has been suggested that these play a crucial role in the pathogenesis and persistence of rheumatoid inflammation.

Patients and methods

The study comprised 37 patients, 35 suffering from oligoarticular juvenile idiopathic arthritis (JIA) and 2 (both males) suffering from enthesitis-related arthritis (ILAR classification) (4) who had been consecutively referred to the Rheumatology Day Hospital Department of the Pediatric Clinic of the Burlo Garofolo Children's Hospital, Trieste from September 1993 to February 1999.

Indications for intra-articular injection of the knee were arthritis at onset which failed to respond after two months' treatment with NSAIDs, and relapse of arthritis after a period of full remission.

One mg/kg (maximum 40 mg) of triamcinolone hexacetonide (Lederspan, Wyeth) was injected into the upper-outer quadrant of the kneecap following arthrocentesis at least two hours after a local application of lidocaine and prilocaine (Emla, Astra). It was advised that the child be kept at home for the first 24 hours following injection, avoiding physical exertion and carrying weights. In all cases, the non-

steroidal anti-inflammatory drug was discontinued at the time of local treatment.

Disappearance of clinical signs of inflammation in the affected joint was considered as full remission (3), and a response was defined as "good" if there was full remission of at least six months duration (1).

Flow cytometry

The synovial fluid extracted was examined for the presence of B CD5+ and T γ/δ+ lymphocytes by the following method: after counting, the cells were re-suspended in phosphate-buffered saline and phenotypically analyzed on the same day. Immunophenotyping was performed using phycoerythrin (PE)-conjugated anti-CD19 (pan B) and anti-pan-γ/δ (TCR-γ/δ1 which recognizes all γ/δ T cells), monoclonal antibodies and fluorescein (FITC)-conjugated anti-CD3 (pan T), anti-CD5 (T cells, B subset) and anti-CD8 (suppressor/cytotoxic T cells) monoclonal reagent. All these MoAbs were from Becton Dickinson. For staining, samples were washed twice with buffer (HBSS: Hank's balanced salt solution) and the cells were suspended in the same buffer at a concentration of 5×10^5/ml. 100 µl of cell suspension were incubated with 10 µl of a mixture of FITC- and PE-conjugated MoAbs at 4°C for 30 minutes, washed twice and analyzed by flow cytometry (FACScan, Becton-Dickinson, Mountain View, CA, USA).

Ten thousand events were acquired in list mode and analyzed by LYSYS II software (Becton-Dickinson). A gate was set on the lymphocyte population on the basis of scatter properties, excluding debris and other cells. The proportion of double stained CD3+/TCRδ1+, CD8+/TCRδ1 and CD5+/CD19+ was calculated after setting the markers to establish background fluorescence on a control sample stained with mouse isotype antibody PE and FITC conjugated (quadrant markers in fluorescence histograms were set using matched isotype controls).

Immunofluorescence

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on Hep-2 cells, using a commercial kit (Halphadia, Alifax S.p.A., Padua, Italy); for each group of determination, a positive and a negative control serum were included. ANA titers < 1:80 were considered negative. Titers ≥ 1:80 were considered positive (5, 6).

Statistical analysis

Data were analyzed using the chi square test run on Graph Pad Prism software, version 3.00 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com

Results

One or both knees were involved in all 37 patients: there were 26 cases of monoarticular arthritis of one knee, 5 cases in whom both knees were involved, 6 cases in whom additional joints were involved (in two patients, two more joints and in four patients. three more joints were involved; there were no cases of more than 4 joints being involved). The mean age at onset was 3.69 years (range 6 months to 10.7 years); 30 (81%) were females, and 7 (19%) males.

Twenty patients with an average age of 3.2 years were treated within the first 6 months of onset (average time-lapse between onset and first injection: 3.5 months); 17 (average age at onset 4.5 years) were treated within a month after a relapse in the same joint, sometimes after long remissions (average remission duration 33 months). A total of 87 injections of 0.7-1 mg/Kg of triamcinolone hexacetonide (Lederspan, Wyeth) were given.

Twelve patients were given a single injection; the remaining 25 received an average 3 injections each.

The 37 patients in the study were followed up for an average of 31.3 months from the time of injection (range 7-65 months) and had a mean remission time of 13.9 months (range 0-54 months). 21 out of 37 (56.7%) were ANA positive, with titers ranging from 1:80 to 1:640. No significant variations in ANA titers were found during the follow-up in individual patients (no more than one dilution). During follow-up, no patients became ANA negative.

Twelve patients (32.5%) had full remission after a single injection and no relapse during follow-up (mean follow-up time of 41.8 months, range 26-69). Thirteen patients (35%) had more than 6 months' remission after local treatment but subsequently had a relapse at varying time intervals (average 19.4 months, range from 6 to 58.8 months). Another 12 patients (32.5%) had a relapse in the same joint within six months of injection (average 3.4 months) and subsequently repeated local treatment at the same amount as the first injection or were started on medical therapy.

The features of these three groups of patients with different response to local treatment are described in Table III.

We observed a similar incidence of uveitis in the ANA positive patients who showed full remission after a single injection (4 out of 7) and in the ANA positive patients who relapsed within six months (6 out of 11). Only in one case was there no positive response to the injection, and the signs and symptoms of local inflammation remained unchanged.

The response was good in 25 patients (67%): they either had stable remission, or had no relapse for at least another 6 months. Furthermore, the data would seem to indicate that prolonged remission over 6 months is more likely if a patient is ANA negative ($p \leq 0.01$ see Table I). Patients ANA positive are equally likely to have a relapse in less than 6 months or not.

Remission time in relation to timeliness of treatment

If we restrict our consideration to timeliness of treatment, the comparison between remission time in patients treated within 6 months of onset and in

Table I. Remission time in relation to ANA after the first injection.

	ANA+	ANA-
Remission > 6 months (n=25)	10	15
Remission < 6 months (n=12)	11	1
chi square = 6.59 $p \leq 0.01$		

those treated subsequently appears statistically significant (see Table II).

The prognosis for ANA+ patients differs according to whether the patient received early or late treatment. Patients who underwent injections soon after onset fared well regardless of ANA (specifically, 8/11 ANA positive and 9/9 ANA negative had remission of more than 6 months). On the other hand, ANA+ patients who underwent injections late seemed to respond worse to local treatment (8/11 ANA+ had a relapse in a very short time compared to 1/6 ANA-).

Remission time in relation to age at onset

There was no correlation between age at onset and response to local treatment. Average age at disease onset in patients with a response time of more than 6 months was 3.44 years, versus 4.15 years for patients who had a relapse within 6 months.

Side effects

In 3 patients (10%), subcutaneous lipolysis resulted in sunken or fallen skin overlying the injected area, due to spillage into the subcutaneous tissue of the corticosteroid injected. There was spontaneous regression of the lipolysis in all cases during follow-up.

Immunological parameters

With regard to the assessment of the percentage of γ/δ lymphocytes on synovial fluid, a total of 31 samples were evaluated from 18 patients, and it was found that percentages varied from 0.5% to 20.1%, with an average of 9.27%, not unlike the percentages found in the peripheral blood of normal subjects (normal range 5 - 15%).

There was no difference in the γ/δ percentage between patients in remission for over 6 months and those who had had a relapse within 6 months (respectively 9.7% versus 8.8% on average). Indeed, if we consider the 6 patients with high γ/δ (over 15%) in synovial fluid, 4 had a stable remission while 2 had a relapse within 6 months.

As regards the B CD5+ lymphocyte population, the average percentage in synovial fluid from 28 samples (for a total of 16 patients) was 0.98% (range 0.5-3%), practically the same as in the serum of normal subjects.

Assessment of T γ/δ and CD5+ lymphocytes was carried out in only 6 cases in successive arthrocenteses. In none of these cases there were significant variations in the lymphocyte subpopulations studied.

Discussion

Local treatment with triamcinolone hexacetonide has modified the therapeutic approach to patients with JIA, greatly reducing recourse to non-steroidal anti-inflammatory drugs and consequently their side effects.

Our study indicates that the earlier the treatment, the easier it is to obtain a lasting and possibly permanent response. Thirty percent of the patients have had no more relapses, and they were all treated within the first six months of disease onset. On the other hand, patients who received late local treatment have prolonged remission, especially if ANA negative. In this study these antibodies, which generally do not appear to be related to the development of arthropathy, are related to a worse prognosis, i.e. there is an increased likelihood of relapse, most probably facilitated by an immunologically more active disease.

In a large case study of patients with seropositive RA, McCarty *et al*. (7) reported stable remission in 75% of the joints treated, which were subsequently splinted for 3 weeks following injection. However, very few studies are available of pediatric patients. The results of the study carried out by Honkanen *et al*. (3) seem to be better than the results reported in this article, but we underline that the former study only considered patients in whom the disease was of less than 6 months' duration.

Other authors (8) studied a group of 21 adolescents who underwent MRI both before and 7 weeks after local treatment, and documented its effectiveness in reducing both inflammation and synovial pannus. Correlations with laboratory parameters have recently been reported by Ravelli *et al.* (1), who have demonstrated that patients with a higher ESR are more likely to benefit from intra-articular glucocorticoid injection of the knee.Yet there are no studies into specific immunologic parameters on synovial fluid in correlation with treatment response, apart from the aforementioned study by Honkanen *et al.* (3), which showed that the higher the percentage of polymorphonuclear cells in the synovial fluid before steroid injection, the shorter the remission time.

With regard to the study of the lymphocyte sub-populations, previous authors reported an increased percentage of B CD5+ and/or T γ/δ+ in blood or synovial fluid from subjects with

Table II. Remission time in relation to timeliness of treatment after the first injection.

	20 patients treated within 6 months from the onset	17 patients treated during a relapse [* 33 months]
Remission > 6 months	17 (8 ANA +)	8 (3 ANA +) [* 35 months]
Remission < 6 months	3 (3 ANA+)	9 (8 ANA+) [* 32 months]

chi square = 4.46 $p \leq 0.03$

* median time from onset of the disease to the first injection

Table III. Features of the different groups of patients as regards to response to local therapy

	N°	Female	Male	ANA +	ANA -
Total patients	37	30	7	21	16
Patients with long lasting remission	12	11	1	7	5
Patients relapsed after 6 months	13	11	2	3	10
Patients relapsed within 6 months	12	8	4	11	1

RA onset in adulthood (9-11). The significance of γ/δ lymphocytes is, however, still uncertain. They play a role in the early phase of immunologic defense against invasive micro-organisms and might also be activated against "stressed" cells which express on their surface Heat Shock Proteins sharing some epitopes with autoantigens (12). This would explain their activation in auto-immune diseases. Moreover, a recent study suggests that the activity of steroids in autoimmune diseases may rely on the susceptibility of gamma delta T-cells to steroid-induced apoptosis (13).

In contrast, with regard to B CD5+ lymphocytes, which are physiologically appointed to produce polyclonal and multi-specific antibodies against auto-antigens, in patients with adult RA these lymphocytes undergo a highly specific monoclonal expansion, possibly correlated with the production of rheumatoid factor (9)

The data on synovial fluid in this study did not show an increase in T γ/δ+ or B CD5+ lymphocytes. The values can be superimposed on those found in peripheral blood and there is no significant difference in terms of probability of remission. These results do not support the hypothesis that they might play a role in the pathogenesis of articular chronic inflammation in JIA as was postulated in adults.

It should also be pointed out that the only side effect of local treatment documented both by our study and others (1,2,14,15) was skin atrophy at the injection site. In our series, the incidence was 3.6%, slightly higher than that of other studies; atrophy occurred in all three cases subsequent to the first injection. This type of lesion generally has a spontaneous, slow resolution, as in our cases, but it can be avoided through manoeuvres to avoid spillage of corticosteroids into the surrounding joint tissue, as some authors have pointed out (1).

In conclusion, we suggest that since this therapy is safe, it should be started early on in the course of disease, since this provides increased likelihood of prolonged remission and avoids long-term treatment with NSAIDs.

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