

TREATMENT WITH LEFLUNOMIDE IN RHEUMATOID ARTHRITIS DECREASES THE LEVELS OF ANTI-CCP ANTIBODIES

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REZUMAT

Objective: evaluarea eficacității pe termen lung a tratamentului cu Leflunomid asociat cu antiinflamatoare nesteroidiene (AINS) în diferite stadii de poliartrită reumatoidă (PR) la pacienții cu forme active de boală și influența asupra nivelului anticorpilor anti-CCP. **Material și metode:** au fost studiați 60 pacienți timp de 2-4 ani: 24 pacienți (40%) - 4 ani, 29 pacienți (48,3%) - 3 ani, 7 pacienți (11,6%) - 2 ani; pacienții se aflau în diferite stadii de evoluție ale PR (stadiul II - 35%, stadiul II/III - 50%, stadiul III - 10%, stadiul III/IV - 5%). Examenul clinic a fost realizat pe baza criteriilor ACR 20, în plus luându-se în considerare și durata redorii matinale. Analizele de laborator efectuate au fost: hemoleucograma, VSH, CRP, FR, anticorpi anti-CCP, teste hepatice. Pe radiografie s-a luat în calcul scorul Larsen-Dale. **Rezultate:** cei mai mulți pacienți au fost din mediul urban (68%), cu forme seropozitive (95%), vârsta fiind cuprinsă între 50-59 ani (73,33%), iar debutul în urmă cu 5-10 ani (66,7%). A fost înregistrată o îmbunătățire a parametrilor inflamatori cu scăderea VSH (41%), CRP (39%), la majoritatea pacienților FR s-a negativat, nivelele de anticorpi anti-CCP au scăzut (58,85%), s-a redus numărul articulațiilor dureroase și tumefiate (peste 75%) precum și durata redorii matinale (50%). La 7 pacienți (11,66%) s-au înregistrat reacții adverse minore. **Concluzii:** Leflunomidul asociat cu AINS a ameliorat simptomele din PR, a oprit progresia modificărilor radiologice, a scăzut FR până la normalizare, a redus nivelele anticorpilor anti-CCP. Toleranța a fost bună. **Cuvinte cheie:** poliartrită reumatoidă, Leflunomid, anticorpi anti-CCP, factor reumatoid.

ABSTRACT

Objectives: The assessment of effectiveness on long term of treatment with Leflunomide associated with AINS in different stages of RA on patients with active forms of the disease and the influence on anti-CCP antibodies. **Material and methods:** 60 patients were studied for 4 years: 24 patients (40%) - 4 years, 29 patients (48.3%) - 3 years, 7 patients (11.6%) - 2 years in different stages of evolution of RA (stage II - 35%, stage II/III - 50%, stage III - 10%, stage III/IV - 5%). The physical examination was performed based on ACR 20 criteria and included the duration of morning stiffness. The following laboratory assays were performed: blood cell counts, ESR, CRP, RF, the antiCCP-antibodies levels, liver tests, radiological exam with Larsen-Dale score. **Results:** Most of the patients were from urban area (68%), with seropositive forms (95%), age of 50-59 years (73.33%) and disease duration was between 5 and 10 years (66.7%). There was an improvement of inflammation parameters with decrease of ESR (41%), CRP (39%), in most of the patients RF values became negative, decrease of anti CCP-antibodies levels (58.85%), number of painful and swelling joints (over 75%), improve of morning stiffness (50%). 7 patients (11.66%) had minor adverse reactions. **Conclusion:** Leflunomide associated with NSAIDs decreased the symptoms of RA, stopped the progression of X ray-changes, decreased RF even to its normalization and antiCCP-antibodies levels. The tolerance was good.

Key Words: rheumatoid arthritis, treatment, Leflunomide, antiCCP-antibodies, rheumatoid factor

INTRODUCTION

Joint damage in rheumatoid arthritis (RA) leads to functional impairment and poor life quality. In order to avoid these consequences, prompt remissive treatment in the early stages of the disease is mandatory.^{1,2}

Although pathogenic mechanisms in RA are still unclear, recent clinical and molecular biological studies have identified a combination between immune cellular subsets, cellular surface markers and soluble cellular products, which play an important role in the inflammatory process in RA. Inflammation and the subsequent synovial tissue degeneration are triggered by the influx of lymphocytes (B cells, CD₄⁺ and CD₈⁺) in the synovial tissue. The activation of the T cells in RA has focused the attention towards a therapy that may regulate the proliferation of these cells. Such a regulating agent is Leflunomide [N-(4-trifluoromethylphenyl)-5-methylizoxazol-4-cardozamide], a synthetic derivate of izoxazole with low molecular weight (~270). The interest for Leflunomide as anti-rheumatic drug is motivated by its unique ability to regulate the

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progression in the cellular cycle by inhibiting de novo pyrimidine synthesis. The active metabolite of Leflunomide, A77 1726, in small therapeutic doses, reversibly inhibits dihydroorate dihydrogenase, the decisive step in the de novo synthesis of pyrimidine. Unlike other cells, activated lymphocytes increase their pyrimidine content approximately eight times during proliferation, the purine quantity increasing about twice. For this to happen, lymphocytes have to use their both ways of synthesis. The inhibition of dihydroorate dihydrogenase by A77 1726 prevents the accumulation by lymphocytes of too many pyrimidines in order to determine DNA synthesis. In large doses, A77 1726 inhibits the tyrosine kinases responsible for the early pointing out of T and B cells. Because the immune regulating effects of A77 1726 become manifest at doses which inhibit dihydroorate dihydrogenase but not tyrosine kinase, the interruption of de novo pyrimidine synthesis can be the main course of action. Recent studies have shown that the anti-inflammatory effects of A77 1726 might be linked to their ability to suppress IL 1 and TNF alpha selective, before their inhibition by the activation of the contact between T lymphocytes and monocytes.^{3,4} It has been demonstrated that A77 1726 inhibits the activation of the nuclear factor kB, a possible mediator of the inflammation, if stimulated by anti-inflammatory agents. Research in progress have shown that A77 1726 can decrease the regulation of molecular adhesion glycolysis, reducing intercellular contact during inflammation.⁵

At the site of the inflammation, the inflammatory cells and the cells of the native tissue are close, which suggests that a possible mechanism of intercellular communication is by direct intercellular contact, associated with the contribution of soluble factors. Some of the studies on the cellular contact between T lymphocytes and monocytes have revealed that intercellular contact induces the production of matrix metalloproteinases (MMPs) and of tissue-inhibiting metalloproteinase (TIMP-1).⁶ Besides, the direct contact determines the regulation of pro-inflammatory cytokines (IL1 and TNF α) and of their inhibitors (IL 1 receptor antagonist and TNF soluble receptor). It has been assumed that an imbalance between MMPs and TIMPs and between cytokines and their inhibitors may lead to the destruction of the matrix, a fact which characterizes chronic inflammation. A77 1726 inhibits the capacity of T cells to stimulate monocytes. At the same time, the active metabolite of Leflunomide inhibits IL 1 β , TNF α , nitric oxide and stromelysin [metalloproteinase 3 (MMP-3)].⁷⁻⁹

The aim of the study is to assess the long-term

effectiveness of Leflunomide associated with NSAIDs in various stages of the disease, in patients with active stages of the disease.

MATERIAL AND METHODS

The study group consisted of 60 patients observed for up to four years. Follow-up period was 4 years in 24 patients (40%), 3 years in 29 patients (48.33%) and 2 years in 7 patients (11.66%). The patients were in various evolutive stages of the disease: stage II – 21 patients (35%); stage II/III – 30 patients (50%); stage III – 6 patients (10%); stage III/IV – 3 patients (5%). The patients received a loading dose of 100 mg/day, followed by a maintenance dose of 20 mg/day.

Assessment was conducted monthly. Clinical examination was conducted by ACR 20 criteria, and the following signs were noted: painful and swollen joints, the duration of morning stiffness. Lab measurements consisted of full blood count, ESR, C reactive protein, rheumatoid factor (RF), concentration of anti-cyclic citrulinated peptide antibodies (anti-CCP Abs) determined using ELISA, liver tests, synovial liquid exam, joints X-rays with Larsen-Dale score.

In order to assess anti-CCP antibodies, the patient's diluted serum is incubated in recipients with highly-purified CCP (2nd generation). Specific serum antibodies are fixed by the immobilized antigen and produce a photometric color reaction through an enzyme fixed by a secondary antibody. Five serum calibrations show an adequate measurement of the concentrations. EUROIMMUN anti-CCP ELISA is a high-sensitivity and specificity serological test for the diagnosis of RA. Anti-CCP antibodies belong predominantly to IgG and have a specificity of 97% for RA, greater than that of the rheumatoid factor (62%). These can be assessed in the early stages of the disease in 79% of the patients.^{10,11}

We used the Larsen-Dale Index, which uses a set of standard films for each joint and distinguishes five stages of involvement:

- I. Absence of alterations;
- II. Swelling of the soft parts (55% of patients);
- III. Juxta-articular osteoporosis (50% of patients);
- IV. Narrowing of joint space (10% of patients);
- V. Bone destruction (5% of patients).

RESULTS

Most patients belonged to urban background (68%); seropositive forms were predominant (95%); the predominant age-group was 50-59 years (73.33%)

and most of them were diagnosed 5 to 10 years earlier (66.7%).

First assessment was carried out at six months in 20 patients; second assessment was carried out at the end of the study

During the treatment period inflammatory tests were decreased: ESR was reduced with 41%, C reactive protein with 39%, and rheumatoid factor became negative in most patients. (Fig. 1) Furthermore, the concentration of anti-CCP antibodies decreased by 53.85%, there were fewer painful and swollen joints (> 75%) and a reduced duration of morning stiffness (with 50%). (Figs. 2, 3).

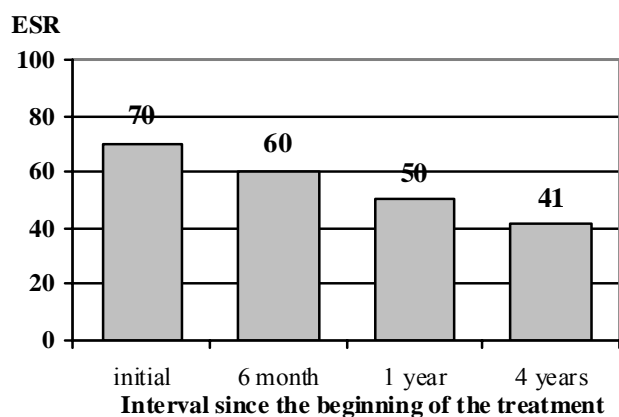


Figure 1. Decrease of ESR.

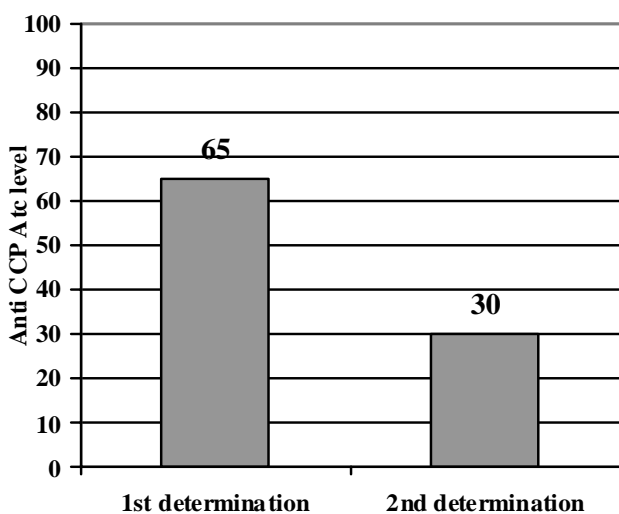


Figure 2. Reduction of anti-CCP antibody concentration.

The clinical improvement defined by ACR 20 was significant for Leflunomide (20 mg/day). At the same time, Leflunomide treatment led to a major improvement of the quality of life and functional ability.

Four patients (6.66%) did not respond to the treatment.

Seven patients (11.66%) presented minor adverse reactions (rash, stomatitis, dyspepsia, weight loss,

increased blood pressure). No major problems occurred regarding anemia, leucopenia or thrombocytopenia. No infections developed in the study period.

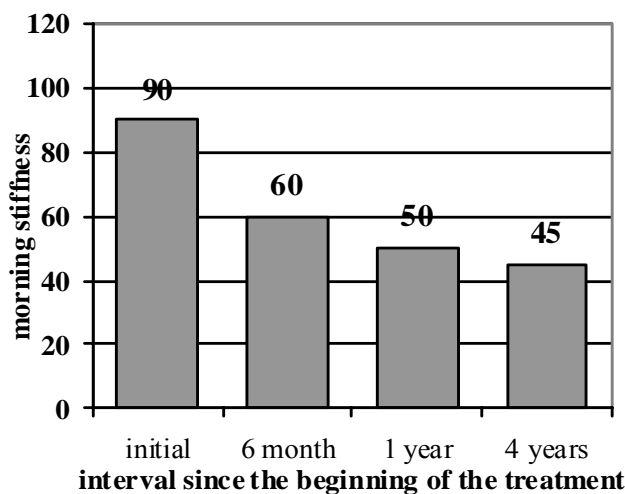


Figure 3. Shorter morning stiffness.

DISCUSSIONS

Anti-CCP antibodies significantly prevailed in patients with severe radiological alterations, similar to previous publications.^{12,13} Recently, Visser described a predictive pattern which includes the assessment of anti-CCP antibodies and which can discriminate between limited, persistent non-erosive and persistent erosive arthritis.¹² The role of anti-CCP antibodies is that of early diagnosis and prognosis, being important for the elaboration of an adequate therapeutic strategy leading to the prevention of articular alterations.¹⁴⁻¹⁸

The present study revealed a significant decrease of the anti-CCP antibodies concentration and of the rheumatoid factor in the serum of the patients treated with Leflunomide. The relatively high prevalence of patients with anti-CCP antibodies and initial positive rheumatoid factor (88% and 95%, respectively) reflects the selection of patients with more aggressive forms of the disease that resisted treatment with other disease-modifying antirheumatic agents (DMARDs). This confirms indirectly the association of anti-CCP antibodies with a more severe progress of the disease.

The presence of anti-CCP antibodies predominated in patients with a high Larsen-Dale score, representing a predictive factor for radiological alterations and their development.¹³ The use of anti-CCP antibodies in clinical practice helps making the appropriate therapeutic decision.^{19,20}

This study also confirmed the long term efficacy and safety of leflunomide in patients with rheumatoid arthritis.

CONCLUSIONS

1. Leflunomide associated to NSAIDs reduced RA symptoms and synovial inflammation.

2. Radiological damage did not progress during the study period.

3. The concentration of the rheumatoid factor decreased and it even became negative in most patients.

4. The decrease of the concentration of anti-CCP antibodies was connected to the regression of the disease, namely to the improvement of clinical symptoms and stopping the progress of the disease, being important for the early diagnosis and having a greater specificity than that of the rheumatoid factor.

5. Tolerance of Leflunomide was generally good, which makes possible its use as first-choice DMARD.

6. The effectiveness of Leflunomide increased as the disease stage the treatment was initiated was less advanced.

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