LETTER TO THE EDITOR

EFFICACY AND SAFETY OF ANTI-TNF-α THERAPY COMBINED WITH CYCLOSPORINE A IN PATIENTS WITH RHEUMATOID ARTHRITIS AND CONCOMITANT HEPATITIS C VIRUS INFECTION

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This study further expands our previous observation demonstrating the usefulness of combination therapy of anti-TNF-alpha and Cyclosporine A in the treatment of rheumatoid arthritis and concurrent hepatitis C virus infection, as well its efficacy and safety in controlling HCV viremia and liver toxicity. Seven patients were included in the study; transaminase levels remained unchanged, HCV RNA serum levels decreased significantly and DAS 28 significantly improved after twelve month follow-up. No side effects were registered.

Chronic hepatitis C virus (HCV) infection is frequently encountered in rheumatic patients. In particular, 0.65-5.4% of patients with rheumatoid arthritis (RA) have been identified as carriers of the HCV ([1]). This condition may represent a challenge for the physician because it is well-known that corticosteroids or cytotoxic drugs may stimulate HCV replication and deteriorate liver conditions.

Cyclosporine A (CsA) is an immunosuppressive agent used to treat autoimmune disorders including RA and there is enough evidence in the literature showing that it also exerts antiviral effects, both *in vitro* and *in vivo*, as demonstrated in liver transplanted patients ([2]). The inhibitory effect on HCV replication of CsA is highly specific and it is exerted by inhibition of cyclophilin B and not by the inhibitory effect on calcineurin, which is responsible for the immunosuppressive effect of CsA. Despite this important information, very few patients affected by concomitant autoimmune disorders and HCV infection, treated with CsA, have been reported in the literature ([3]).

Anti-TNF-α blockers are immunosuppressant agents used to treat severe RA. Post-marketing analyses have clearly demonstrated an increased susceptibility to infections in patients under anti-TNF-α treatment. Therefore, inhibition of TNF-α by anti-TNF-α treatment, in RA patients with concomitant chronic HCV infection, may cause adverse events which can significantly change the clinical course of both diseases.

Nevertheless, clinical studies on the use of anti-TNF-α in HCV-infected RA subjects have demonstrated their usefulness and safety, with particular regard to HCV viremia and liver toxicity ([4]- [9]). In addition, information about the combination

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therapy with CsA and anti-TNF-α agents in this condition are limited to our previous report ([10]). The favourable outcome of this therapy in the first two RA cases, in terms of both safety and efficacy, encouraged us to extend the treatment to other patients.

Herein, we report preliminary data of the first multicenter, prospective, open study on the combined therapy with CsA and anti-TNF-α agents in patients with concomitant RA and chronic HCV infection. Primary end-points were a) to assess the efficacy of this therapy on RA, and b) to verify its safety on liver function; the secondary end-point was to confirm the ability of CsA to decrease HCV serum viral load also in RA patients who contemporarily take anti-TNF-α agents.

MATERIALS AND METHODS

Seven patients, five females and two males, mean age 57.5±13.1 years, were consecutively enrolled in the study. RA was diagnosed according to the American College of Rheumatology (ACR) criteria and HCV infection was defined by a positive HCV viral load. All patients were negative for anti-HIV antibodies, hepatitis B surface antigen and did not report a significant alcohol consumption. Informed consent to participate in this study was obtained for all prospective patients. Therapy with anti-TNF-α and CsA was started at the same time. At baseline each patient underwent an abdominal sonography. Time-points of interest were baseline and every month for laboratory investigation and rheumatic assessment, while viremia was monitored every three months. The observed variations of HCV viral load were considered significant when at least ±50% of the baseline value was found ([11]). Biology follow-up included blood cell count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), rheumatoid factor (RF), erythrocyte sedimentation rate, C-reactive protein, cryoglobulin determination, antinuclear antibodies (ANA) detected using HEP-2 cells (INOVA), anti-cyclic citrullinated peptide antibodies (anti-CCP) by ELIA system (Phadia). Genotyping was determined on all HCV RNA-positive specimen. Viral load was performed by reverse transcriptase polymerase chain reaction (RT-PCR). Twenty-eight tender and swollen joints according to the Disease Activity Score (DAS) were recorded. The follow-up data referred to twelve months. A value of $p < 0.05$ was considered statistically significant (Wilcoxon test).

RESULTS

At the beginning of combined therapy, the mean ± SD duration of RA was 152 ± 121.4 months and the mean ± SD duration of HCV infection was 97 ± 52.5 months.

All patients had RF positive, and anti-CCP were reported in four patients. ANA and cryoglobulins type III were positive in two subjects. The viral genotypes are reported in Table I. All patients had been treated with DMARDs prior to starting combined therapy. These drugs included methotrexate (MTX), leflunomide (LEF), sulfasalazine (SSZ) and hydroxychloroquine (HCQ). They were discontinued either for poor disease control or for adverse events, including liver toxicity in some cases. Four patients were given etanercept at a dose of 25 mg twice a week, three patients were treated with adalimumab at a dose of 40 mg every two weeks. All started CsA at a dose of 3mg/kg/day and continued to take low doses of 6-methyl prednisolone and/or NSAIDS. One patient reduced the dose of etanercept to 25mg/weekly because of neutropenia. Two patients reduced the dosage of CsA to 50 mg bid, because of increased creatinine level and increased blood pressure after four and five months of therapy, respectively. No patient received any specific antiviral therapy. Clinical and serological baseline and follow-up data are presented in Table I. Cumulative data analysis in all patients at the end of follow-up showed a significant articular and serological improvement of RA symptoms; in particular, DAS 28 from the mean basal value of 5.27 ± 0.38 reduced to 2.98 ± 0.63 (decrease of 43.45%, p=0.0156). In addition, a value of DAS 28 < 2.6 was recorded in two patients.

Regarding erythrocyte sedimentation rate, it is worth noting that its mean value was 45 mm/h at baseline and decreased to 29 mm/h at the end of follow-up (decrease of 35%; p = 0.05) (data not shown). Furthermore, no significant variations of liver enzymes (alanine aminotransferase - ALT: mean values from 37.86 ± 23.49 IU/ml to 26.43 ± 6.27 IU/ml; decrease of 30.18%; p= n.s.) (aspartate aminotransferase - AST: mean values from 41.71 ± 30.24 IU/ml to 24.71 ± 3.04 IU/ml; decrease of 40.75%; p= n.s.) were observed at the end of follow-up. HCV viral load decreased by 67.16% of baseline value (mean values from 7.11×10^6 to 2.34×10^6 IU/ml; p=0.0781); viremia increased in only one patient in the absence of relevant hepatic clinical manifestations.

Table I. *Demographic and biological characteristics of patients.*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (yrs)	65	54	31	72	65	58	58
Sex	F	M	F	F	F	M	F
Previous DMARDs	HCQ, MTX	MTX	MTX, LEF	SSZ, MTX, LEF	LEF	MTX	MTX, SSZ
AR duration months	101	384	156	240	60	66	60
RF	Positive	Positive	positive	positive	positive	positive	positive
Anti-CCP	Positive	Negative	positive	positive	negative	positive	negative
ANA	Positive 1:160 speckled	Negative	positive 1:320 homogeneous	negative	negative	negative	negative
Anti-TNFα treatment	Etanercept	Adalimumab	Etanercept	Etanercept	Adalimumab	Etanercept	Adalimumab
HCV duration months	104	94	72	90	204	84	32
Genotype	2c	3a	1a	2c	2a	2c	1b
Viral load (IU/ml): baseline	2.7x10^7	2.8x10^6	1x10^7	1.2x10^6	2.4x10^6	5.5x10^6	6.2x10^6
Viral load: end of follow-up	7x10^6	1.2x10^6	1.8x10^6	6.5x10^5	2.7x10^6	1.6x10^6	2.8x10^5
Cryoglobulins	Positive type III	Negative	positive type III	negative	negative	negative	negative
ASAT (IU/ml): baseline	22	26	47	20	34	36	107
ASAT: end of follow-up	24	26	23	20	27	19	28
ALAT (IU/ml): baseline	18	18	47	10	47	49	76
ALAT: end of follow-up	18	18	32	10	29	28	27
DAS 28: baseline	4.96	4.8	5.57	5.13	5.71	5.04	5.71
DAS 28: end of follow-up	2.37	3.15	1.88	3.12	3.26	3.33	3.76

DISCUSSION

Many studies have reported CsA as having a substantial antiviral activity, besides the well-known immunosuppressive action. This peculiar effect of CsA has been widely noted in the treatment of liver transplanted patients and of patients affected by autoimmune disorders and concomitant HCV infection (2).

We have recently demonstrated the safety of CsA in 32 patients with autoimmune disorders and concomitant HCV infection, including 25 RA individuals ([12]). This study also confirms the ability of CsA to significantly reduce the HCV viral load *in vivo*. Furthermore, elevated TNF-α levels have been documented in patients with HCV and there is evidence showing an association between high TNF-α levels and a worse prognosis of liver damage. In addition, it has been postulated that the pathogenesis of hepatocyte destruction in chronic HCV may be mediated by the up-regulation of inflammatory cytokines, including TNF-α.

To date, there are no guidelines adequately assessing the use of anti-TNF-α in patients with chronic infections, such as HCV. On the other hand, despite the few case reports in the literature, anti-TNF alpha agents seem to be safe also in HCV patients affected by autoimmune diseases, without worsening the liver outcome. In this regard, in a retrospective study on 31 RA patients with chronic HCV infection, we demonstrated the safety and efficacy of anti-TNF-α agents in this condition (11).

In the present prospective, open study we treated seven RA-HCV positive patients with CsA and anti-TNF-α after clinical failure with various DMARDs. The therapy was well-tolerated and was

also efficacious in controlling articular symptoms, as proven by the decrease in the DAS 28 score. Concerning safety, serum aminotransferase levels remained normal over all the period of observation; in addition, in one patient with high basal values, we noticed their normalization in time. Concerning the viral load, it is well-known that substantial variation of viremia in few subjects can be related to the possible analytical variation in HCV RNA measurements and/or to spontaneous biological fluctuation. In our case, the cumulative viral load decreased significantly, reaching 67.16% of the baseline value at the end of the study. Moreover, it is notable that these effects were the same independently of the HCV genotype.

Our preliminary experience shows the efficacy and safety of the CsA - anti-TNF-α therapy in RA patients. Finally, we demonstrated a reduction in the viral load under CsA treatment, even in the presence of anti-TNF-α therapy. The advantage of adding CsA would be to better control viremia, which remained unchanged or increased slightly in patients treated with anti-TNF-α alone (11).

Further advantages for combining CsA with TNF-α blockers in RA, from the beginning of therapy, could be represented firstly by the proven ability of CsA in reducing TNF-α secretion and secondly, by the indirect inhibition of B-lymphocytes in producing antibodies directed against anti-TNF-α agents. In conclusion, combination therapy CsA - anti-TNF-α agents can be considered a good therapeutic option in RA patients also affected by chronic HCV infection. Further controlled studies on larger samples are required to confirm our preliminary results.

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