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Cyclosporine A: good response for patients affected by autoimmune disorders and HCV infection?

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Abstract. – *Introduction:* **In autoimmune disorders (ADs), if Hepatitis C Virus (HCV) is present, immunosuppressive treatment could increase virus replication. Cyclosporine A (CsA), in standard therapeutic doses, has been proven able to inhibit HCV cyclophilin in vitro. Therefore CsA could improve the therapy of HCV patients with ADs.**

Aim: **In these patients, we started an open pilot study to evaluate the safety of 3 mg/kg CsA and the ability to reduce steroid therapy.**

Patients and Methods: **Five females and 1 male were recruited; mean age 66 ± 8 years, mean disease duration 13 ± 5 years. Three patients are affected by Psoriasic Arthritis, 1 by Rheumatoid Arthritis, 1 by Sjögren Syndrome, and 1 by Myasthenia Gravis. None of them had chronic active hepatitis. HCV genotypes were type 2 (in 3 cases) and type 1 (in 3 cases). Patients were treated with 3 mg/kg of CsA for a period of time ranging from 6 to 12 months. The starting mean dose of prednisone was 12.5 mg/day. Liver function tests were checked monthly and serum HCV-RNA load was checked by RT-PCR before and 2 months into the therapy.**

Results: **The prednisone dose was reduced from 12.5 mg/day to 7.5 mg/day. The aminotransferases levels were unchanged after 6 months. In patients with low HCV-RNA levels before treatment, no modifications of viral load were observed, whereas patients with increased levels at onset showed mild reduction 2 months into the treatment.**

Conclusions: **Immunosuppressive treatment of ADs patients with HCV infection can be safely provided with the integration of CsA.**

Key Words:

Cyclosporine, Autoimmune disorders, HCV infection, Autoantibodies, interferon.

Introduction

Hepatitis C virus (HCV), an enveloped positive-stranded RNA virus of the genus *Hepacivirus* and family *Flaviviridae*, is an important cause of morbidity and mortality in worldwide[1]. A high proportion of individuals infected develop chronic hepatitis[1]. In western countries the prevalence of HCV infection is about 3% while in south Italy there are areas where the prevalence rises up to 20% of the population[ii]. Since autoimmune disorders (AD) have a relatively high prevalence (about 10% of population), in daily clinical practice patients with both AD and HCV infection are usually present[2]. Moreover, if laboratory investigation are carried out in HCV affected patients, it may reveal a various spectrum of autoantibodies, including rheumatoid factor, anti-cardiolipin antibodies, smooth muscle antibodies (SMA) anti-parietal cell antibodies (APCA), anti-liver/kidney microsomal antibodies type I (ALKMA1) and anti-neutrophil cytoplasmic antibodies (ANCA)[3-5]. On the other hand, chronic HCV infection may be responsible for several autoimmune or immune-mediated conditions[6] such as rheumatoid arthritis[7], mixed cryoglobulinemia[4], panarteritis nodosa[4], sicca syndrome[4], hepatitis C virus-related arthritis (HCVRA)[8], psoriatic arthritis[9], and myasthenia gravis[10]. Other rheumatologic manifestations reported in patients with chronic HCV infection include fibromyalgia, systemic lupus erythematosus (SLE), antiphospholipid syndrome, and osteosclerosis[10]. Various mechanisms were proposed to explain the association between HCV and autoimmunity. Out of these, the issue

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of HCV-related autoimmunity has partly been shown to be related to the resistance of CD5+ B cell subpopulation to apoptosis[3].

It is also well-known that immunosuppressant agents and/or glucocorticoids, usually used in the treatment of AD, can increase viral replication and consequently may worsen the clinical evolution of HCV infection[11-12].

For those reasons, physicians have often refrained from using these drugs in AD patients, who also affected by HCV infection, especially when HCV-RNA is present[2].

The development of effective immunosuppressive therapies, which don't lead to the increase of serum HCV-RNA levels, remain the largest challenges in the near future. Recent (*in vivo* and *in vitro*) studies have showed that Cyclosporine A (CsA), which is used for many years in organ transplant patients, do not increase HCV-RNA levels but it is even able to inhibit HCV replication, such as antiviral drug[12-13]. These same effects are also seen using non-immunosuppressive derivative of CsA: the NIM811[1]. For understanding mechanisms of action of CsA it need to remember structural organization of HCV genome (Figure 1).

Structural Organization of the HCV Genome

The HCV genome is a 9.6 kb single-stranded RNA of positive polarity. It encodes for a large polyprotein that, following maturation results in at least 10 proteins: the structural proteins C, E1, E2 and p7 and the non-structural proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B (Figure 1). The viral RNA dependent RNA polymerase is encoded by NS5B[14].

CsA

CsA is a calcineurin inhibitor, and acts directly blocking the transcription of IL-2. This cytokine drives the T cell proliferative response, which is a prominent factor for the propagation of the inflammation process[15]. In particular, CsA binds to a group of proteins called cyclophilins (CyPs). The CsA-cyclophilin A (CypA) complex abolish the phosphatase activity of calcineurin, which is critical for the expression of the cytokines and their receptors in T cells, thus blocking T-cell activation[12].

CsA: Anti-HCV Activity

There are sufficient evidences suggesting that CsA exerts antiviral effects being able to inhibit replication of several viruses, including human immunodeficiency virus (HIV), hepatitis B virus (HBV), and HCV replication in vitro[17].

Teraoka et al. reported also that CyA had a beneficial effect in two chimpanzees chronically infected with HCV The mechanism of action for CsA to suppress HCV replication is only beginning to be understood[11]. The calcineurin pathway and the immunosuppressive function overall do not appear to be involved in the CsA-mediated suppression of HCV as FK506 does not have any inhibitory effect and CsA derivatives without immunosuppressive function can still inhibit HCV replication very effectively. Recent evidence supports the hypothesis that one or more cyclophilins (probably cyclophilins B9, B10, "CypB") are involved in the replication of HCV in vitro by serving as a cofactor for NS5B, the viral RNA-dependent RNA polymerase. CsA suppress HCV replication by disrupting the association of CypB with NS5B[11,15]. This is in line with observations of Fernandes et al[18], who showed that different sets of mutations in NS5A and NS5B, conferred different levels of cyclosporine resistance.

The antiviral effects of Cys A has also been confirmed *in vivo*, in liver transplanted patients and in patients affected by chronic active hepatitis (CAH)[8].

Effects on Different Six Genotypes of HCV

There are six major HCV genotypes with a number of subtypes that vary by geographic distribution and mode of transmission: 1a, 1b, 2a, 2b, 3a, 4a[19]. It is reported that CsA can induce reductions in HCV-RNA levels in a replicon cell

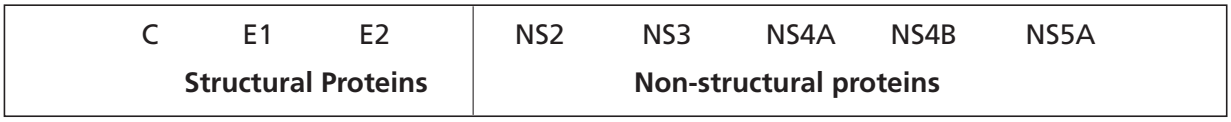

Figure 1. Structural organization of HCV genome.

culture system using HV-1b and HCV-4a, but its ability to inhibit a HCV-2a genotype virus is reduced[1]. These observations indicate that different HCV genotypes are differentially susceptible to CsA.

Efficacy and Safety of CsA

Nevertheless, very little is known about the efficacy and safety of CsA in patients with autoimmune disorders and concomitant chronic HCV infection but preliminary studies suggest that CsA can be safely used in the treatment of patients affected by autoimmune disorders with concomitant chronic HCV infection[2,8].

We report personal experiences with CsA in the treatment of autoimmune disorders in the presence of chronic HCV infection.

Patients and Methods

Six subjects have been recruited in our study. Five patients were females and one male, mean age was 66 ± 8 years and mean disease duration (time of diagnosis) was 13 ± 5 years. Three patients were affected by Psoriasic Arthritis, 1 by Rheumatoid Arthritis, 1 by Sjögren syndrome, and 1 by Myasthenia Gravis. None had CAH. HCV genotypes were 2 (in 3 cases) and 1 (in 3 cases).

Patients have been treated with 3 mg/kg of CsA for a period of time ranging from 6 to 12 months. The starting mean dose of corticosteroids (prednisone) was 12.5 mg/day and after 6 months it was tapered to 7.5 mg/day.

Liver function tests were checked every month and serum HCV-RNA load was checked by RT-PCR before and 2 months during therapy.

Results

After 6 months, serum aminotransferase levels have showed no modifications during CsA treatment. No modifications of viral load have been seen in patients who had low RNA levels before treatment, whereas patients with increased levels of Hepatitis C virus-RNA at onset have showed mild reduction 2 months after the treatment. Notably the mean prednisone dose significantly reduced after 6 months of therapy from the initial 12.5 mg/day to 7.5 mg/day.

Discussion

Autoimmune disorders and HCV chronic infection frequently occur in the same patient. The treatment of AD may become difficult due to the well-known risk to worsen the HCV infection outcome with corticosteroids and/or immunosuppressants.

Cyclosporin has been used for many years in transplantation. Other fields of application merit to be investigated, including the treatment of chronic hepatitis C virus (HCV) infections and autoimmune disorders like autoimmune hepatitis[20]. Firstly we report the recent evidence of anti-HCV effect of Csa; in the end we describe the use of this immunosuppressive agent in patients affected by HCV infection and autoimmune disorders.

Regarding to *HCV chronic infection* present therapies are based on interferon (IFN)-α and ribavirin[21], with a success rate around 35-40% of patients treated (this percentage changes in relation to genotype and other factors). Thus the development of new therapeutic agents is a high priority goal.

In 2003 CsA was reported to be clinically effective against HCV infection[22].

Nakagawa et al[23] also showed that cyclosporin A was able to specifically inhibit HCV replication in a dose-dependent manner. No changes in the rate of cell growth or in viability were observed, indicating that the inhibition was specific and was not a general cytotoxic effect. No specific inhibitory effects were seen with either tacrolimus or rapamycin. The maximum effect of CsA in terms of inhibition of HCV replication *in vitro* is similar to that obtained with IFN-α and the effects of these two agents are certainly additive, and possibly synergistic.

Treatment of the cells with both cyclosporin A and IFN-α produced a greater decrease than using either agent alone[24]. Moreover a controlled trial showed that the combination of CsA with Interferon is more effective than IFN monotherapy[25]. The virological response at both 12 and 48 weeks was significantly higher in patients treated with the combination therapy than with IFN a alone. ALT normalized at 48 weeks in significantly more patients receiving combination therapy. In patients with HCV genotype 1 infection, the sustained virological response was markedly higher in patients receiving combination therapy than in those receiving IFN-α alone.

Cyclosporin is widely used in *transplantation*. One of the major causes of liver transplantation is decompensated cirrhosis due to chronic hepatitis C. It would be interesting to determine whether CsA (as immunosuppressive therapy after liver transplantation) delays or prevents recurrence of HCV in comparison with other immunosuppressive treatments.

Ghobrial et al[26] retrospectively analysed the time to second orthotopic liver transplantation in 71 patients who required this procedure. Of the patients in whom graft failure was due to recurrent HCV infection, patients who received CsA after transplantation had a longer time interval between the first and second transplant than patients treated with tacrolimus.

Levy et al[27] looked at the incidence of biopsy-proven acute rejection in 495 transplant recipients randomized to treatment with cyclosporin A or tacrolimus with corticosteroids and with or without azathioprine. Graft loss or death at 6 months occurred in significantly fewer HCV-positive patients treated with cyclosporin than treated with tacrolimus, while in HCV-negative patients there was no difference.

Pollard et al[28] showed that a greater proportion of HCV-positive patients who received cyclosporin A after liver transplantation had a significantly lesser degree of fibrosis progression. Regarding to autoimmune disorders (AD), it is not rare to encounter patients with AD also carrying HCV[2]. Chronic HCV infection may be responsible for the appearance of several autoimmune or immune-mediated conditions[29] and it is not rare to encounter patients affected by autoimmune disorders who also carry HCV. These situations are very similar to those found in liver transplanted patients, because patients contemporary receive corticosteroids and/or immune-suppressants (to treat the autoimmune disorders) and antiviral agents (to treat the viral infection)[2].

The use of steroids or immunosuppressive drugs in HCV infected individuals is considered a risk for worsening the clinical outcome of HCV infection, so that rheumatologist have often refrained from using these drugs in AD when HCV-RNA is also present. The evidence that CsA exerts an inhibitory effect on HCV replication at standard therapeutic dose has opened new ways to improve the therapy and the prognosis in patients with HCV-related liver diseases.

Miura et al[30]. described four patients affected by dermatological disorders (nummular dermatitis, psoriasis vulgaris, psoriasis pustulosa, pruriago nodularis) and HCV infection treated with 3 mg/kg daily of CsA. The Authors showed that long-term administration of CsA (from 24 to 36 months) was safe and reduced HCV load and aminotransferase levels in three out of four patients.

Galeazzi et al[31] conducted a pilot study in 7 individuals to evaluate the safety and the antiviral effect of CsA in patients with ADs and concomitant HCV infection. Patients were affected by HCV-related symptomatic mixed cryoglobulinemia, Sjogren syndrome, rheumatoid arthritis and chronic autoimmune hepatitis. Patients were treated, for a period of time ranging from 6 to 12 months, with 3 mg/kg of CsA and glucocorticoids at the mean dose of 17.5 mg of prednisone. Liver function tests and serum HCV-RNA load were controlled. After 6 months serum aminotransferase levels significantly decreased in two patients with chronic autoimmune hepatitis and no modifications were seen in the others who had normal levels before treatment. Hepatitis C virus-RNA load significantly decreased in four patients who had higher levels before treatment. No modifications of viral load were seen in patients with low RNA levels before treatment. It has been very interesting to observe that in one patient with rheumatoid arthritis and concomitant 1b HCV infection with CAH, the HCV-RNA test resulted negative after 8 months of CsA therapy, and was still negative after 2 months. In the same patient serum aminotransferases slowly returned to normal limits and remained normal over time. Mean prednisone dose was significantly reduced after 6 months of therapy from the initial 17.5 mg/day to 12.5 mg/day.

Thanks to this study, the Authors confirmed the safety of CsA in the treatment of AD with concomitant HCV infection and they suggested the safety of cyclosporine in the presence of all HCV genotypes even in patients with high viral load.

We reported below the experience with CsA in patients affected by rheumatoid arthritis and autoimmune hepatitis. Tumour necrosis factor-α (TNF-α) is a key cytokine in the integrated host defence system against infectious diseases. Rheumatologist are refrained to use anti-TNF-α in patients with autoimmune disease and concomitant chronic HCV infection. Recently a study of 24 patients with chronic HCV infection and rheumatoid arthritis treated with anti-TNF therapy (etanercept or infliximab) didn't show significant adverse events[32].

Galeazzi et al[2] described two patients affected by rheumatoid arthritis treated with anti-TNF and CsA. In one case, Adalimumab (Humira) was given at the standard dose of 40 mg every 2 weeks subcutaneously, in the second Etanercept (Enbrel) was administrated subcutaneously at the dosage of 25 mg twice a week. These patients have been treated for 6 months and no detectable side effects were seen so far. Aminotransferases remained within normal limits and viral load did not increase.

Autoimmune hepatitis (AIH) usually shows a good response to high dose prednisone and azathioprine treatment. Nevertheless more than 15% of patients (in particular young patients or those with a long history of disease before treatment) develop a primary treatment failure and a significant number of patients needs withdrawn from the treatment due to important side-effects. A relapse after discontinuation of therapy can be as high as 75%[33]. Cyclosporin A (CsA) has been studied as a possible drug in AIH because directly blocks the transcription of cytokines, IL-2 in particular, which drive the T cell proliferative response. Unlike most of the other immunosuppressive drugs, CsA does not induce bone marrow suppression, does not suppress other stem cells and thas a rapid onset of action. The use of cyclosporin in autoimmune hepatitis is not well documented. However, preliminary results from small series of patients or case reports seem to point out a possible role for CsA in the treatment of AIH.

Malekzadeh et al[34] evaluated the effectiveness of cyclosporin both in patients already treated with steroids and in patient who had not received any previous treatment. Patients received a cyclosporin A dose of 2-5 mg/kg body weight/day in two divided doses and were followed up for 26 weeks. The mean levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased significantly over the 26 weeks of the study. Serum creatinine levels did not change significantly. The treatment was well tolerated and preferred by some patients to their previous corticosteroid regimens. According to the authors CsA could be considered as an alternative first-line therapy for autoimmune hepatitis. Nevertheless, despite the encouraging results from this study, the advantages of CsA therapy over conventional treatments remain unclear, due to the lack of a randomized comparison with the conventional treatment arm and because 26 weeks of therapy may be a too short period to develop significant long-term toxicities.

Fernandes et al[35] reported a remission after 3 months of therapy with cyclosporin A, 2-3 mg/kg body weight/day in two divided doses in four patients with type 1 AIH.

Sherman et al[36] also described five patients who achieved not only a remission after 10 weeks of therapy with cyclosporin A, 3-6 mg/kg body weight/day, but also an histological improvement in terms of reduction of necroinflammatory activity.

In an open label, multinational, multicenter trial performed in 32 children with definite AIH, CsA was administered alone for 6 months (target trough levels 200-250 ng/mL), followed by low doses of prednisone and azathioprine for 1 month, after which CsA was discontinued. Among the 30 children completing the course of treatment, 25 achieved clinical and biochemical remission by 6 months and the other 5 by 1 year[37]. Adverse effects of CsA were mild and disappeared after drug withdrawal.

In another open label study[38], 15 children with type 2 AIH were treated with CsA. In all subjects, ALT activities normalized within 6 months with minimal side effects. No relapse occurred in 10 patients after 1 to 6 years. Two children presented with acute liver failure unresponsive to standard therapy and showed a dramatic response to CsA with normalization of clotting parameters. The authors concluded for an improved toxicity profile compared to the side effects encountered with 1 to 2 mg/kg/day of induction prednisone.

The adverse events associated with the use of CsA have been observed in a larger study and defined mild by the authors, including hypertrichosis, gingival hyperplasia (39%), creatinine elevation (9.5%) and hypertension[39].

The use of CsA as a salvage therapy is a reasonable alternative to prolonged high-dose corticosteroids and it certainly deserves further clinical investigation to be administered as first-line therapy in autoimmune hepatitis.

Conclusions

CsA can be safely integrated in immunosuppressive treatment of ADs patients concomitantly affected by HCV infection. Autoimmune disorders and HCV chronic infection frequently occur in the same patient and the treatment of AD may become difficult due to the well-known risk to

worsen the HCV infection outcome with corticosteroids and/or immunosuppressants. The experiences in transplanted patients clearly show that a potent immunosuppressive regimen prevents allograft rejection but it also favours viral replication, facilitating viral-mediated graft injury. Recent studies *in vitro* and *in vivo* demonstrated that CsA has substantial antiviral activity. Thus CsA can act with a double action: (1) decreasing the activity of the immune system, CsA avoids graft rejection in transplanted patients and reduces autoimmunity damage in AD patients; (2) CsA interferes with virus replication.

Even if further prospective trials are required to confirm the efficacy and safety of CsA in HCV+ individuals, the available data are highly suggestive that the use of CsA contributes to better outcome also in patients affected by AD and concomitant HCV infection.

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