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Treatment with peg-interferon alfa-2b and ribavirin of hepatitis C virus-associated mixed cryoglobulinemia: a pilot study ☆

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***Background/Aims*: The aim of this study is to verify the efficacy and safety of peg-interferon alfa-2b in combination with ribavirin for initial treatment of HCV-associated mixed cryoglobulinemia.**

***Methods*: Eighteen patients (7 women and 11 men) affected by mixed cryoglobulinemia were included in the study and treated with peg-interferon alfa-2b 1.0 μg/kg once a week plus ribavirin (1000 mg daily) for 48 weeks, regardless of the HCV genotype.**

***Results*: At the end of the treatment HCV-RNA became undetectable in 15 patients (83%) and most patients improved clinically. One subject suspended treatment at 13th week due to depression. A large fraction of the patients (8 cases: 44%) relapsed both virologically and clinically a few weeks after the end of therapy. At the end of follow-up, only eight patients (44%) obtained a sustained virological response.**

***Conclusions*: Peg-interferon alfa-2b in combination with ribavirin seems safe and useful for patients affected by mixed cryoglobulinemia, but not as effective as in patients with HCV-positive chronic hepatitis without cryoglobulinemia.**

***Keywords*: Hepatitis C virus; Peg-interferon; Ribavirin; Mixed cryoglobulinemia; Vasculitis**

1. Introduction

Hepatitis C virus (HCV) determines not only chronic liver disease, but is also implicated in several lymphoproliferative disorders [1–5], among them the most common is mixed cryoglobulinemia (MC). The treatment of MC includes several drugs like steroids [6], cyclosporins [7], colchicine [8], plasmapheresis [9] and others [10], but given the documented association with HCV virus, the treatment of choice seems to be the antiviral therapy [11–15]. For chronic hepatitis C, until recently the interferon alfa plus ribavirin was the standard of care [16], but the development of pegylated interferons opened new treatment opportunities [17], even for MC. The aim of this pilot study was to verify safety and efficacy of peg-interferon alfa-2b in combination with ribavirin for initial treatment of HCV-associated MC.

2. Patients

Eighteen patients (7 women and 11 men) affected by MC were included in the study. None of the patients had received antiviral therapy in the past (naïve). Diagnosis was based on standard criteria, and showed an active disease, defined as recurrent purpura associated with constant arthralgias and fatigue, despite steroid therapy. In addition to purpura, weakness and arthralgias, eight patients (44%) showed Raynaud's phenomenon, one had

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'sicca syndrome', three had peripheral sensitive neuropathy (17%) and one nephropathy. All patients were Caucasian, heterosexuals and had no history of intravenous drug abuse or ethanol abuse. All patients gave their informed consent before entry into the study, which had been previously approved by the ethic committee of the Region Friuli-Venezia Giulia. In this open-label pilot study, the quality of life was also assessed with the EORTC QLQ-C30 instrument [18].

3. Methods

Values for the liver function tests as well as hematological parameters were determined by usual laboratory methods. Rheumatoid factor (RF), C3 and C4 fractions of complement were measured by rate nephelometry. Cryoglobulin determination was performed according to standard methods as previously reported [19]: the cryoprecipitates, diluted in 0.5 M NaCl, were fractionated by high-resolution gel electrophoresis to type cryoglobulins. Individual monoclonal bands were identified by immunofixation after electrophoresis using a cellulose acetate strip impregnated with antibodies specific for heavy and light chains. Mixed cryoglobulins were classified as type II on the basis of the presence of monoclonal IgM immunoglobulins with RF activity complexed with polyclonal IgG, and as type III in the presence of polyclonal immunoglobulins.

3.1. Purpura scoring system

To assess the severity of vasculitis, the following clinical scoring system was used. A score of 0 indicated the absence of skin lesions; a score of 1, the presence of less than 10 purpura spots on the lower leg; a score of 2, the presence of more than 10 spots on the lower leg; a score of 3, the extension of the spots to the upper leg and/or the abdomen; and a score of 4, the presence of skin ulcers and/or gangrene.

3.2. Histology

A liver biopsy was obtained in all but one patients. Samples were placed in buffered formalin, stained with haematoxylin and eosin, and, for reticulum, with Gomori stain. In each biopsy the disease activity and fibrosis were assessed according to METAVIR [20].

3.3. Virological studies

Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) markers were detected by enzyme-linked immunosorbent assay (ELISA) using commercially available kits. The presence of anti-HCV antibodies was assayed by the second generation (four-antigen) immuno-enzymatic screening test ORTHO-HCV (Ortho Diagnostic Systems, Raritan, NJ, USA). The presence of HCV-RNA in the serum was assessed by nested PCR amplification of the conserved 5′ untranslated region (5′UTR) of HCV. The HCV genotypes were determined with the line probe assay (Inno-Lipa HCV; Innogenetics, Zwijnaarde, Belgium).

3.4. Therapy

Since these patients had a different immunological situation from subjects carrying chronic hepatitis C without CM, we decided to treat all subjects, regardless of HCV genotype, as follows:

- peg-interferon alfa-2b (PegIntron, Schering-Plough,) 1.0 μg/kg once a week (QW) for 48 weeks plus
- ribavirin (Rebetol, Schering-Plough) 1000 mg/daily for patients with body weight 65–85 kg, or 1200 mg for those above this weight, for 48 weeks.

We decided to implement the bucket dosing recommendations for peg-interferon alfa-2b schedule [21], and to use a lower weight-based dosage of 1.0 μg/kg in order to reduce adverse events and to improve the compliance.

3.5. Criteria for therapy evaluation

To overcome the difficulties in finding homogeneous criteria to evaluate different aspects of the disease, as indicated in our previous reports [22], the response to treatment was split into four separate categories: (1) virological response, (2) biochemical response, (3) immune response, and (4) clinical response.

(1) *Virological response*: effect of treatment on HCV-RNA. Sustained virological response (SVR)–complete response: loss of HCV-RNA at the end of follow-up. Relapse: loss of HCV-RNA at the end of treatment but positivity at the end of follow-up (6 months). No response: persistent positivity during therapy and at the end of follow-up.
(2) *Biochemical response*: effect of therapy on ALT; normal value for the local laboratory was considered 53 IU/l. Complete response: normalization of the serum ALT level during treatment followed by normal ALT values lasting for 6 months after discontinuation of therapy. No response: ALT out of normal value during treatment and follow-up. Relapse: normalization of the serum ALT level during treatment followed by return to abnormal values during follow-up. In some patients this parameter was not considered, since the ALT level was normal at the beginning of the treatment.
(3) *Immune response*: effect of therapy on serum RF concentration and cryocrit level; normal value for RF was considered as 0–30 IU/ml. Complete response: normalization of serum RF concentration and disappearance of circulating cryoglobulins. Partial response: reduction (but not normalization) of RF and cryoglobulins $\geq$50%. No response: Reduction $<$50% of RF and cryocrit levels or stable levels. Relapse: partial or complete normalization of serum RF and cryoglobulins during therapy followed by return to higher values during follow-up.
(4) *Clinical response*: effect of therapy on the clinical manifestations of the disease (including purpura, arthralgia and weakness). Complete response: disappearance of all clinical signs of the disease. Partial response: improvement of the clinical symptoms (reduction of the purpura score $\geq$50%). No response: reduction of the purpura score $<$ 50% or stable disease. Relapse: partial or complete normalization of clinical symptoms during therapy followed by return to higher score after the end of treatment.

3.6. Follow-up

Biochemical and clinical parameters were determined each month during therapy and every 2 months during follow-up, up to 6 months. Auto-antibodies were determined every 3 months and thyroid function tests every 6 months. Determinations of HCV-RNA were performed before starting the therapy, at 12th week, at the end of treatment (EOT) and at the end of follow-up (EFU). All patients were followed for at least 6 months after the end of therapy.

3.7. Statistical analysis

Descriptive statistics were performed, including proportions, means and standard deviations (SD) of relevant variables.

4. Results

4.1. Biochemical and histological findings

The main clinical, laboratory, and histological findings of patients are captured in Table 1. The age of patients is expressed as years at the start of therapy (mean age 50 ± 11 years). In 3 subjects (17%), no monoclonal component was found; accordingly, in these 3 cases the mixed cryoglobulinemia was defined as type III. A liver biopsy was performed in all patients but one. A chronic liver disease of variable severity was found in all subjects (see Table 1).