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Abstract Type II mixed cryoglobulinemia is sustained by an oligoclonal production of IgM sharing rheumatoid activity and can be associated with renal, cutaneous, rheumatologic or neurological manifestations. Peripheral neuropathy is a major cause of morbidity in hepatitis C virus-associated mixed cryoglobulinemia and is often refractory to any treatment. Rituximab induces a selective depletion of IgM-producing B cells, and both case reports on monoclonal IgM-related polyneuropathy as well as studies on small series of patients with interferon a-resistant mixed cryoglobulinemia have suggested that it may be beneficial. Thirteen patients affected by type II mixed cryoglobuline- mia with polyneuropathy were treated. Rituximab was administered intravenously at a dose of 375 mg/m2 on days 1, 8, 15 and 22. Two more doses were given 1 and 2 months later. No other immunosuppressive drugs were added. Response was evaluated by assessing the changes in the clinical neurological condition, in electromyographic indi- ces and in laboratory parameters (including cryocrit, viral load, complement levels and rheumatoid factor) over at least 12 months. Sensory symptoms disappeared or improved following treatment. A significant improvement in the clinical neuropathy disability score was observed. Electro- myography examination revealed that the amplitude of compound motor action potential had increased. Viral load did not significantly change. Side effects were negligible. In this open prospective study, rituximab appeared to be effective and safe in the treatment of patients with type II cryoglobulinemia-associated neuropathy.

Keywords HCV-related mixed cryoglobulinemia Á Cryoglobulinemic peripheral neuropathy Á Rituximab

Introduction

Mixed cryoglobulinemia is an immunological disorder characterized by immune-complex-mediated systemic vasculitis involving small vessels, which may present with renal, cutaneous, rheumatologic and/or neurological manifestations.

The reported incidence of peripheral neuropathy in MC patients varies from 7 to 100% [10, 33, 38]. The patho- genetic mechanism associated with cryoglobulinemic neuropathy is thought to be either ischemic, i.e, an obstruction of the vasa nervorum by intravascular cryo- globulins, or secondary to a vasculitic process of the vasa nervorum [10, 25]. A close relationship between cryo- globulinemia and chronic hepatitis C virus (HCV) infection is suggested by the detection of circulating anti-HCV antibodies and a high HCV RNA concentration in cryo- precipitate [31].

Interferon a is reportedly effective in treating hepatitis and in reducing the clinical manifestations of cryoglobu- linemia [5]. However, the improvement is generally temporary [12], and the response is mild in patients with neuropathy [11, 39]. Moreover, during acute immunological flare-ups, antiviral treatment is usually either insufficient to control vasculitic manifestations [36] or may even be det- rimental, albeit able to reduce viremia [6]. Steroids and immunosuppressive drugs (usually cyclophosphamide), and on occasion plasmapheresis, are advocated in these cases [29], even though improvement in neuropathic symptoms is inconstant. Rituximab, a chimeric monoclonal antibody directed against the CD20 protein, was successfully used in the treatment of IgM-related polyneuropathies [19]. These effects were recently confirmed in a discrete cohort of patients collected in a multicenter study [4], though a few unresponsive [28] or even worsened cases [8] were also reported. Some patients with cryoglobulinemic vasculitis and peripheral neuropathy were also treated [32, 35, 37]. However, the possible confounding effects of the associated therapies, especially steroids, did not allow the investigators to draw any definitive conclusion on the specific role of Rituximab in peripheral neuropathy.

Peripheral neuropathy is a major cause of morbidity in MC. This makes the issue of the actual effectiveness of Rituximab in MC- associated polyneuropathy especially relevant. The effects of Rituximab in 13 patients with cryoglobulinemic neuropathy are reported in the present study.

Methods

Patients and study protocol

This was an open, prospective, uncontrolled study on patients previously unsuccesfully treated with more current therapies, including corticosteroids in 11 cases, immuno- suppressive agents in 4 (cyclophosphamide in 3 and mycophenolate mofetil in 1), plasma exchange in 3 and interferon (in 4 cases).

Clinical features and previous treatments (which were discontinued at least 3 months before Rituximab adminis- tration) of the 13 patients under study are summarized in Table 1. Evaluation included clinical history, physical examination, electrophysiologic studies and serum testing.

Laboratory studies included serum chemistry profiles, immunoglobulin and complement levels, erythrocyte sed- imentation rate (ESR), plasma C reactive protein (CRP), rheumatoid factor and cryoglobulin determination.

Each patient gave written informed consent. Rituximab was intravenously administered at a dose of 375 mg/m2 on days 1, 8, 15 and 22. Two more doses were administered 1 and 2 months later, according to a previous protocol that had been successfully employed in patients with severe cryoglobulinemic nephritis [32]. Premedication included oral antihistamines, acetaminophen (500 mg) and deflaza- cort (a single, 60-mg administration upon first infusion). No steroids or other immunosuppressive drugs were given in association with Rituximab.

Complete neurological examination was performed prior to treatment and then every 6 months during the 12 months of follow-up using a clinical neuropathy disability score (CNDS). A slightly modified CNDS score as described by Mariette and co-workers was used [23]. Briefly, selected items from the neurological assessment were scored and summed. The strength of four muscles (92) was scored according to the Medical Research Council of Great Britain scale in six grades, from 0 (complete paralysis) to 5 (normal strength); four muscle stretch reflexes were scored as 1 if present and 0 if absent; pain, paresthesia (hand and foot), dysesthesia, burning foot and sensory ataxia were scored as 1 if absent and 0 if present. Gait was scored as 1 if normal and 0 if abnormal. Asthenia was scored as 0 if present and 1 if absent. Scores could range from 0 to 60 (i.e., normal neurological eval- uation). In addition, the patient was asked to rate the change in paresthesia using a visual analogue scale (VAS).

Electrodiagnostic analysis

Electrodiagnostic studies were performed before and after 12 months during follow-up using standard electromyo- graphic equipment (Medelec Synergy SYN-T2 Oxford Instruments, Old Woking, Surrey, England). Skin temper- ature was maintained at 36°C throughout all the nerve

conduction studies. Motor nerve conduction studies were performed by supramaximal percutaneous nerve stimula- tion. Compound muscle action potential (CMAP) was recorded by using surface electrodes. Motor conduction velocity, distal motor latencies and amplitude of CMAP (baseline to negative peak) were measured in the peroneal nerve on both sides. Sensory nerve conduction velocity and amplitude sensory nerve action potential (SNAP) were measured in the sural nerve on both sides. SNAPs were recorded by using surface electrodes.

Cryoglobulin detection

Cryoglobulin determination was performed as follows. Venous blood was collected in a warm syringe then transferred into warmed tubes and stored at 37° C until it clotted. After centrifugation at 37°C, the serum was col- lected and stored at 4°C for 1 week. Warm syringes were systematically used for all testing. After isolation and washing, the cryoprecipitate was quantified and expressed as a percentage of precipitate/serum volume (i.e., cryocrit). The cryoprecipitate components were

characterized by immunofixation electrophoresis [33] using a commercially available kit that was sensitive enough to detect the pres- ence of oligoclones (Criokit, Helena Labs, Milan, Italy).

Determination of HCV genotype and viral load

The HCV RNA sequence was tested by PCR using using 50 untranslated (50UTR) nested primers [13]. HCV genotypes were identified using nested PCR amplification with type- specific primers of the NS5 region and of the core. Viral load was determined using a signal that was amplified through branched DNA (bDNA, Quantiplex, HCV RNA 2.0 Assay; Chiron Co., Emmerville, CA) [13].

Statistical analysis

The results were expressed as means ± SD. Continuous variables were compared by Student's t test. Categorical variables were evaluated using the v2 test. All statistical analyses were performed using GraphPad Prism software version 4 (GraphPad Software, San Diego, CA).

Results

Nine women and four men were enrolled. Each patient had type II, i.e., IgMk/IgG cryoglobulins. Twelve out of 13 were infected by HCV (Table 1). Paresthesias were present in 11 patients, while 6 complained of burning feet and 1 had restless legs syndrome. Asthenia was present in 12 patients. After treatment, six patients still had paresthesias, two had burning feet, and two had asthenia. Prior to treatment, patient no. 7 was unable to walk. He began to walk again following the third infusion. Prior to treatment, the mean CNDS was 46.08 ± 7.62, while after Rituximab the mean value was 50.23 ± 7.17 (P \ 0.001) (Table 2).

Viral load was determined in 11 HCV positive patients and was found to have decreased in 7 cases, increased in 3 patients and was unchanged in 1 (Table 3).

The electrophysiological data were consistent with an axonal neuropathy. Twenty-six peroneal nerves were studied in order to record motor response. Before treat- ment, CMAP was absent in 5 out of 26 peroneal nerves, while a reduced amplitude (\3 mV) of motor response was present in 21/26 peroneal nerves. After treatment, motor response was present in all patients, and the amplitude of CMAP was normal ([3 mV) in 4/26. The mean value of CMAP amplitude significantly improved after treatment from 1.24 to 2.03 mV (Table 4). The motor conduction velocity significantly improved from 33.89 m/s to 41.26 (Table 4). With regards to the distal motor latency, no significant differences were observed following therapy. SNAPs of the sural nerve were absent in 11 patients at entry. After treatment, two patients regained sensory response (P \ 0.002). No relationship was found between duration of symptoms and response to therapy.

Discussion

Until recently, MC had not been considered a frequent cause of peripheral neuropathy. MC represents a unique model of interaction between an infectious trigger, i.e., the hepatitis C virus, and an immune-mediated disorder [26]. It has been estimated that about 170 million people world- wide are infected with HCV, with a seroprevalence rate of 1% in Western Europe, but as high as 3% in some Medi- terranean areas. Cryoglobulinemic neuropathy is probably the most common form of vasculitic neuropathy in Italy [15]. In the largest survey of cryoblobulinemic patients with nephritis ever published, which included about half of all the patients recorded in the Italian Registry of Renal Biopsies in 1995, peripheral neuropathy was found in 9.7 percent of cases at disease onset, but in 28.7 percent of patients at the last follow-up (mean 6.7 ± 3.6 years, range 1-16 years) [33]. However, when peripheral neuropathy was systematically searched for in a single reference cen- ter, it was found in all 19 patients who underwent electrophysiological examination during follow- up [33]. Peripheral neuropathy is thought to be the result of axonal ischemic damage caused by deposits of cryopreci- pitable immune complexes in the vasa nervorum. Nevertheless, high levels of antineuronal IgG (anti-GM1 and anti-sulfatide) were recently detected in MC patients, thus suggesting that direct autoantibody reactions may play an additional role in affecting neuronal structures [1]. Antiviral therapy has often been prescribed to patients with HCV-associated cryoglobulinemia with vasculitis [16, 24, 27]. However, the results of studies involving antiviral therapy in neuropathic patients—mainly consisting of small cohorts—are controversial [2, 17]. De novo appear- ance or worsening of HCV-related cryoglobulinemic neuropathy has been reported within 6 months of interferon therapy [6, 14, 20], even though similar effects were not reported in a prospective study using pegylated interferon [7]. In some instances, the combined treatment of predni- sone (usually 1.0 mg/kg/day) and either oral (1.0-2.0 mg/ kg/day) or intravenous (0.5-1.0 g/m2) cyclophosphamide with plasma exchange (1 plasma volume/session) as esca- lation therapy may be needed [14]. However, these therapeutic strategies can cause a substantial increase in viremia levels, thus exacerbating chronic hepatitis C dis- ease [22]. Rituximab has raised hopes for a new therapeutic approach for patients with manifestations of severe cryo- globulinemic vasculitis [34]. Rituximab is a humanized mouse monoclonal antibody directed at CD20, a B cell- specific membrane protein with four transmembrane- spanning domains that are members of a family that includes Fc gamma R chains. Rituximab induces a selec- tive depletion of IgM- producing B cells [3, 32], and this represents the rationale for the treatment of mixed cryo- globulinemia [32]. Indeed, MC is thought to be the result of oligo/monoclonal proliferation of IgMk-producing lym- phocytes that share rheumatoid activity toward anti-HCV polyclonal IgG. The megacomplexes thus formed are slowly removed from circulation [30, 31], deposit in tissues and obstruct the vasa nervorum.

Rituximab was shown to be effective in several manifestations of vasculitic MC [9, 34, 37]. However, its efficacy in peripheral neuropathy has only been tested in limited studies. The interest of the present study is that drug effec- tiveness was specifically assessed in an open prospective study using Rituximab alone. In our hands, Rituximab ameliored cryoglobulinemic neuropathy that had proved to be resistant to previous treatments. It is noteworthy that a significant improvement in CMAP amplitude after treatment was shown by electromyographic analysis over a relatively short period of time. While these data are consistent with the predominantly axonal feature of cryoglobulinemic neurop- athy, they are in apparent contrast with the current hypothesis that the cryoglobulin involvement of the vasa nervorum invariably leads to complete axonal loss. Some role

in the induction of the neuronal injury in cryoglobuli- nemic patients might be played by episodes of transient conduction block due to either incomplete capillary obstruction or potentially reversible inflammatory pro- cesses. It is likely that the damage with axonal pattern typical of cryoglobulinemic neuropathy, which results from Wallerian degeneration, takes a longer time to occur. Before irreversible axonal damage is established, inflammatory events or partial vessel occlusion by cryoprecipitable immune complexes might sustain potentially reversible conduction blocks that precede the definitive axonal dam- age. Experimentally, when administered immediately prior to animal immunization, Rituximab fully prevents primary and secondary antibody response. This is consistent with the expression of CD20 on naı¨ve and memory B-cells. In cases of established disease, presentation of the antigen by non B-cells may be sufficient to sustain the autoimmune process, especially if autoantibody-producing plasma cells are long lived. Therefore, a standard course of Rituximab might be ineffective in immune-mediated disorders unless combined with agents that target plasma cells [21]. As already observed in mixed cryoglobulinemia-associated glomeru- lonephritis [32, 34], cryoglobulinemic peripheral neuro- pathy represents an immune-mediated condition in which Rituximab specifically targets the cells secreting the monoclonal immunoglobulin involved in the production of cryoglobulins and has proven to be effective, even given alone, in the relatively long run.

Of further interest, the HCV viral load mostly remained stable or decreased during the 12-month follow-up period. These data challenge the idea that B cell depletion induced by Rituximab invariably favors an increase in HCV RNA [35] and confirm the already reported safety of Rituximab in the treatment of HCV-associated cryoglobulinemia [29, 34, 37].

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Conflict of interest statement . None

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