Long-term effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis

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Abstract

Background. Type II mixed cryoglobulinaemia (MC) is a systemic vasculitis, associated in most cases with hepatitis C virus (HCV) infection, and sustained by proliferation of oligoclonal cells. Systemic B-cell depletion and clinical remission can be achieved in non-Hodgkin lymphoma by a human/mouse chimeric monoclonal antibody that specifically reacts with the CD20 antigen (Rituximab). Similar effects could be expected in type II MC.

Methods. Six patients, mean age 64.2 years (range: 37–76 years), with HCV infection genotype 2a2c (three cases) or 1b (three cases) and symptomatic type-II MC with systemic manifestations, including renal involvement (five cases) and bone marrow clonal restriction (three cases), were considered eligible for Rituximab therapy. Rituximab was administered intravenously at a dose of 375 mg/m² on days 1, 8, 15 and 22. Two more doses were administered 1 and 2 months later. No other immunosuppressive drugs were added. Response was evaluated by assessing the changes in clinical signs, symptoms and laboratory parameters for 18 months.

Results. Levels of proteinuria, erythrocyte sedimentation rate and cryocrit significantly decreased at 2, 6 and 12 months. Rheumatoid factor and IgM significantly decreased at 6 months whereas C4 values significantly increased at 2 and 6 months. HCV viral load and immunoglobulin G remained stable. Bone marrow abnormalities were found to reverse to normal in all three positive cases. Constitutional symptoms (skin ulcers, purpura, arthralgia, weakness, paraesthesia and fever) disappeared or improved. No acute or delayed side effects were observed.

Conclusions. Rituximab appears to be a safe and effective therapeutic option in symptomatic patients with HCV-associated MC glomerulonephritis and signs of systemic vasculitis.

Keywords: anti-CD20 monoclonal antibody; glomerulonephritis; Rituximab; type II mixed cryoglobulinaemia

Introduction

The so-called ‘essential’ mixed cryoglobulinaemia (MC) was originally defined in 1966 by Meltzer et al. [1] as a distinct disorder characterized by leukocytoclastic vasculitis with small and, less frequently, medium-sized vessel and multiple organ involvement. Clinical manifestations included chronic hepatitis, arthritis, membranoproliferative glomerulonephritis, peripheral neuropathy, skin ulcers, diffuse vasculitis syndrome and, in some cases, lymphatic and hepatic malignancies [2]. Over the last decade it has become definitely clear that MC is not an ‘idiopathic’ but rather a chronic virus-triggered multisystemic disease, which is strongly associated with hepatitis C virus (HCV) infection [3]. The pathogenetic implication of HCV in the formation, transport and removal from circulation of cryo precipitable immune complexes (ICs) has been studied extensively. ICs are formed by HCV, anti-HCV polyclonal immunoglobulin (Ig) G and monoclonal IgM sharing rheumatoid activity, which is critical for both cryoglobulin production [3] and renal deposition [4]. Due to the IgM component, these cryo precipitable
ICs also escape the erythrocyte transport system [5] and directly impact hepatic and splenic macrophages, which are unable to process them due to abnormalities in the biogenesis of lysosomal enzymes [6]. HCV is lymphotropic [7]. Lymphocytes that are chronically stimulated by HCV are assigned to widespread auto-antibody production related to HCV-induced lowering of the cell activation threshold [7]. Moreover, B cells, protected from apoptosis by an HCV-dependent gene translocation [8], develop oligoclonal monotypic lymphoproliferations. Distinct lymphoid infiltrates with cells expressing oligo or monoclonal rheumatoid factor in the portal tracts, spleen and bone marrow [on occasion evolving towards an overt B-cell non-Hodgkin lymphoma (NHL)] have been described [3]. Therefore, the most important pathogenetic aspects of the disease are (i) abnormal kinetics and easy deposition of HCV containing ICs due to the IgM component, (ii) chronic stimulation by HCV infection sustaining the synthesis of cryoprecipitating IgM rheumatoid factor and (iii) a subclinical smoldering lymphoproliferative disorder.

Based on these considerations, a strong rationale for therapy with anti-CD20 monoclonal antibodies can be envisaged. Rituximab (Matthera-Hoffman-Roche, Milan, Italy) is a chimeric monoclonal antibody that binds to the B-cell surface antigen CD20. Expression of CD20 is restricted to the B lymphocyte lineage. CD20 appears at the late pre-B stage of development and is lost during the terminal differentiation into plasma cells. Therefore, Rituximab may be expected to interfere with monoclonal IgM production, cryoglobulin synthesis and renal deposition of ICs. Monoclonal anti-CD20 antibodies have proven to be highly effective in the treatment of relapsed or refractory low-grade NHL, acting on complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and apoptosis induction [9]. More recently, Rituximab has been used to treat a variety of haematological plasma cell disorders [10–12], including mixed cryoglobulinaemia [13]. Treatment of patients with cryoglobulinaemic glomerulonephritis, however, is very limited. Disappearance of proteinuria was observed in one case with recent onset of nephritis [13]. In another case, the drug was discontinued after two infusions due to acute retinal artery thrombosis [14]. Nephropathy is a major cause of morbidity in MC. This makes the issue of the effectiveness of Rituximab in MC-associated nephritis especially relevant. The present report specifically addressed this issue.

Subjects and methods

Patients

Six patients, consecutively needing a rescue therapy because of unusual bone marrow lymphocyte infiltration (two cases) or either resistance (two cases) or intolerance (two cases) to standard immunosuppressive therapy, were given anti-CD20 monoclonal antibody.

Patient 1. A 76-year-old woman with a 21 year history of inconstant arthralgia and leg purpura. In 1990 she was found to have circulating cryoglobulins. Cryoglobulins remained untyped until 2001, when she was referred from another region to our unit (Centro Multidisciplinare di Ricerche di Immunopatologia e Documentazione su Malattie Rare, CMID), where a diagnosis of HCV (2a/2c)-related mixed IgM-κ/IgG (type II) cryoglobulinaemia with chronic hepatitis was definitively made. She complained of severe weakness, fever, Raynaud’s phenomenon, arthralgia and dysesthesia due to multiple mononeuritis. Proteinuria was 0.3 g/day. Bone marrow examination showed moderate (8%) B-cell infiltrate with clonal IgM-κ restriction, which was compatible with a diagnosis of CD5-negative NHL. She was treated with four cycles of 375 mg/m²/week Rituximab and remained under observation for two more months until re-examination of bone marrow, which proved to be normal. She returned to her region and was lost from follow-up for 16 months. She came back to our observation 18 months later when she was asymptomatic and still had normal bone marrow (Table 1).

Patient 2. A 74-year-old woman with a history of hemi-thyroidectomy (when she was 47 years old), relapsing lumbar and abdominal Herpes zoster infection, diffuse bone deminer-alization, type 2 diabetes (since she was 61 years old) and hypertension. At 63 years of age, she presented with purpura and a diagnosis of type II (IgM-κ/IgG) cryoglobulinaemia was made. Five years later, she was treated with hydroxychloroquine and low-dose prednisone due to relapsing purpura and onset of diffuse arthralgia. At 73 years of age, she presented with signs of heart failure and severe nephrotic syndrome. Thus, diuretics, angiotensin-converting enzyme inhibitors and cyclophosphamide were started. One month later she had fever, leukopenia (1.8×10⁹ white blood cells/l) and a severe episode of pulmonary congestion. She also had bloody stool, which was interpreted as a vasculitic manifestation. She was treated with three pulses of i.v. methylprednisolone, but developed diabetes decompensa-
tion. In the end she was referred to our centre. Besides the diabetes, which required starting of insulin therapy, she also had severe chronic hepatitis due to HCV infection (genotype 2a/2c) with echographic signs of initial cirrhosis, chronic immunogenic thyroiditis, sensitive and motor polyneuropa-thy prevalently involving the legs, haemorrhagic maculopathy (that required discontinuation of hydroxychloroquine) and mild mitral and aortic insufficiency. She also had proteinuria (4.5 g/day) with hypoalbuminaemia (29 g/l), renal failure (creatinine clearance 38 ml/min), diffuse oedema, hyperten-sion, arthralgia, weakness and purpura. Bone marrow examination showed the presence of foci of CD20-positive lymphocytes, including a nodule of 1 cm in diameter. Due to initial cirrhosis, accurate echo-colour Doppler examination was done to rule out arteriolarization of the Retius circle and renal biopsy was performed under desmopressin protection. Renal biopsy findings were consistent with the diagnosis of mixed nephropathy with mesangial changes, which were characteristic of diabetes, and endocapillary hypercellularity and double contours, due to mixed cryoglobulinaemic nephritis. Immunofluorescence and electron microscopy studies confirmed the findings of light microscopy. Table 1 summarizes the short-, medium- and long-term effects on
Table 1. Biochemical data of patients before Rituximab (at admission), 2, 6, 12 and 18 months after therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>sCr (mg/dl)</th>
<th>ESR (IU/ml)</th>
<th>RF (IU/ml)</th>
<th>IgM (mg/dl)</th>
<th>C3 (mg/dl)</th>
<th>C4 (mg/dl)</th>
<th>Cryocrit (%)</th>
<th>Viral load (C × 10^6/dl)</th>
<th>ALT (IU/l)</th>
<th>Proteinuria (g/day)</th>
<th>TBP (g/dl)</th>
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<td>72</td>
<td>101</td>
<td>88</td>
<td>17</td>
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<td>213</td>
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SCr, serum creatinine; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; ALT, alanine aminotransferase; TBP, total blood proteins; ND, not determined.

Viral load was measured by branched DNA and expressed as viral copies/ml.
biochemical parameters of a four-cycle treatment of 375 mg/m² Rituximab followed by 2 more doses 1 and 2 months later.

**Patient 3.** A 67-year-old woman who had a long-term history of necrotizing purpura, leg bilateral polyneuropathy, recently developed nephrotic-range proteinuria (6 g/day) and chronic hepatitis related to HCV (genotype 1b) associated with mixed IgM-/IgG cryoglobulinaemia. She was initially treated with prednisone 1 mg/kg/day and cyclophosphamide 1.5 mg/kg. Two weeks later, the immunosuppressive drug was discontinued due to severe leukopenia and the patient was finally referred to our unit where she underwent kidney biopsy. She was found to have a typical cryoglobulinaemic glomerulonephritis. She was given a bolus dose of 15 mg/kg methylprednisolone, followed by prednison 0.8 mg/kg/day and mycophenolate mofetil 2 g/day. Six months later, she still presented with proteinuria 1.8 g/day, haematuria 3+, severe asymmetric multineuropathy with mixed pattern, i.e. axonal and demyelinating, with both sensitive and motor involve-ment of the arms and legs. Cutaneous ulcers persisted, as did severe weakness and moderate arthralgia. Mycophenolate mofetil was discontinued and 3 months later the patient was administered a 4 + 2 cycle course of therapy with 375 mg/m² Rituximab. Table 1 summarizes the effects on biochemical parameters.

**Patient 4.** A 72-year-old woman with a past history of bone tuberculosis (at the age of 25). Histological diagnosis of cryoglobulinaemic glomerulonephritis was made 10 years prior to treatment with Rituximab. Cryoglobulinaemia had already been typed as IgM-/IgG and was associated with HCV (genotype 2a/2c) chronic hepatitis. She had been treated previously with steroids, interferon and plasma exchange. In 2002 she complained of purpura, worsening of mixed sensory and motor neuropathy, arthralgia, weakness and fever. Urinalysis showed some increase in proteinuria (≤1 g/day) and polymeric haematuria (40 red blood cells/high-power microscopic field). Bone marrow examination showed IgM- clonal restricted lymphocyte interstitial infiltration compatible with a diagnosis of Waldenstrom’s disease. She was administered a 4 + 2 cycle course of Rituximab. Table 1 summarizes the biochemical results.

**Patient 5.** A 59-year-old man who did not undergo biopsy due to haemophilia. In 1997 he developed a nephrotic syndrome with a full clinical picture of mixed IgM-/IgG cryoglobulinaemia associated with HCV infection (genotype 1b). He was treated with steroids, cyclophosphamide and plasma exchange. In 2002, when he was scheduled to receive an arteriovenous fistula in order to start dialysis, he requested consultation in our unit. He had severe renal insufficiency with nephrotic syndrome, four-drug resistant hypertension, four-limb sensitive motor multiple neuritis, arthralgia, weakness, iatrogenic diabetes and purpura. We attempted treatment with a 4 + 2 cycle course of Rituximab (375 mg/m²). Table 1 summarizes the effects of therapy on biochemical parameters.

**Patient 6.** A 37-year-old woman with a history of nodose erythema of legs, who complained of bilateral knee, ankle and hand arthralgia for 2 years and had a 3 week lasting mild fever resistant to antibiotics and purpura. She also had a mild renal failure with proteinuria, haematuria with erythrocyte casts, trace amounts of cryoglobulins, but very low C4 and increased levels of rheumatoid factor, erythrocyte sedimentation rate and C-reactive protein. She was also found to have hepatitis C, with HCV genotype 1b and an incomplete sicca syndrome with xerostomia and low levels of antinuclear and anti-extractable nuclear antigens of SS-A/Ro specificity. Renal biopsy showed features of focal membranoprolif-erative glomerulonephritis with thrombi occluding capillary lumina and interstitial foci of lymphocytes surrounding (and infiltrating) the wall of a pre-capillary arteriole. Steroid therapy was rapidly discontinued because of glucose intolerance. Table 1 summarizes the short- and medium-term effects of a 4 + 2 cycle course of Rituximab (375 mg/m²).

**Symptom scoring**

Paraesthesias and arthralgia were graded according to a patient scored visual analogue scale (VAS). Decreases >25% and >50% in VAS score was assumed as partial or definite responses, respectively.

**Statistical analyses**

The one-way analysis of variance (ANOVA) with Dunnett’s post test was performed using GraphPad Prism version 4.00 for Windows® (GraphPad Software, San Diego, CA).

**Results**

Compared with the initial values, a significant decrease of erythrocyte sedimentation rate, proteinuria and cryocrit was observed 2, 6 and 12 months after first administration of Rituximab (Figure 1). A significant decrease for rheumatoid factor and IgM was also observed at 6 months. C4 significantly increased at 2 and 6 months. IgG levels and viral load did not change. Eighteen month biochemical parameters, available in three patients, were similar to 12 month data (Table 1). Nineteen months following initiation of therapy, patient three complained of relapsing arthralgia and worsening paraesthesia. She had increased rheumatoid factor and dropped C4 levels. Fourteen months after first Rituximab administration, serum creatinine in patient 5 rose to 5.3 mg/dl without other changes in biochemical parameters.

**General symptoms and other parameters**

The beneficial effects of Rituximab therapy on constitutional symptoms included purpura (that disappeared in four out of four cases within 2 weeks), cutaneous ulcers that disappeared in 2 months, weakness and arthralgia that definitively improved, as determined by the VAS scale (>50% decrease), in all six cases, and fever that disappeared in all of the three cases presenting with this symptom. No changes in the electrophysiological studies of neuropathy could be determined. Nevertheless, five patients claimed a definite improvement of paraesthesia. Bone marrow abnormalities were checked again in cases 1, 2 and 4 and proved to have completely reversed to normal.
Fig. 1. Biochemical profiles of six patients with cryoglobulinaemic glomerulonephritis who had undergone a 4+2 cycle treatment with Rituximab (375 mg/m²). RF, rheumatoid factor; ESR, erythrocyte sedimentation rate. *P < 0.05 and **P < 0.01, as calculated with the one-way ANOVA test and Dunnett’s multiple comparison post-test.
Fig. 2. Pathogenesis of cryoglobulinaemic nephritis and rationale for Rituximab treatment. B lymphocytes are targets of HCV infection due to cell expression of the CD81 receptors, which also allow hepatocyte infection [7]. B cells are assigned to widespread autoantibody production related to HCV-induced lowering of the cell activation threshold. A HCV-dependent gene translocation able to protect cells against apoptosis sustains the oligoclonal monotypic lymphoproliferation that occurs in mixed cryoglobulinaemia [8]. The IgM-κ that has rheumatoid activity towards anti-HCV IgG forms mega-complexes that do not bind to the erythrocyte transport system [5], remain free to circulate and saturate the phagocyte's ability to remove ICs from the blood. Phagocyte cell blockade is favoured by HCV infection, which makes cells unable to digest cryoglobulins following phagocytosis [6]. Due to the affinity to the mesangial matrix of the monoclonal IgM component [4], cryoprecipitable ICs deposit in the glomeruli, where cytokine production favours leukocyte diapedesis and endothelial injury. The anti-CD20 monoclonal antibody acts at the very first step of this cascade, blocking B-cell proliferation and, thus, IgM production, which is critical for both cryoglobulin production [3] and deposition in the glomeruli [4].
Side effects

No acute or delayed severe side effects were seen. Pre-
médication included low doses of hydrocortisone, antihista-
mine drugs and acetaminophen. Cardiac and respiratory rate, arterial pressure and temperature were
determined at 30 min intervals. Drug-related brady-
cardia (< 50 beats/min) was observed in two cases and
could be managed by reducing infusion rate. Arterial
pressure lowering until 100/60 mmHg was observed
in one case and was also managed reducing infusion
rate. Patients were also given low doses of aspirin or
analogues to prevent thrombosis [14].

Discussion

The optimal strategy for HCV-associated MC nephritis
is still undefined. Discovery of an association between
MC and HCV and the possible pathogenetic link
prompted clinicians to try to control disease by eradic-
ating the triggering infection. Alpha-interferon treat-
ment has been used increasingly in the past decade.
Results have been inconclusive, despite a few reports of
beneficial effects [15,16]. It is hopeful that some advances
can be expected by the introduction of pegylated interferon, especially in combination
with ribavirin [17].

However, several side effects of IFN therapy have
been reported [18]. Moreover, during acute immuno-
logical flare-ups, antiviral treatment is usually either
insufficient to control renal disease [2], albeit able to
reduce viraemia, or even detrimental [18]. Steroids
and immunosuppressive drugs (usually cyclophospha-
mide) and, on occasion, plasmapheresis, are advocated
in these cases. However, these therapeutic approaches
may cause a substantial increase in the levels of
viraemia, thus, exacerbating chronic hepatitis C disease
[19]. Nevertheless, immunosuppression is still regarded
as the first-line intervention if renal involvement is
severe. The rationale is further reinforced if peripheral
B-cell expansion and lymphoid infiltrates in bone
marrow can be detected. Such infiltrates have been
regarded as ‘early lymphomas’ and are substantially
indistinguishable from small lymphocytic lymphomas
and immunocytomas [8]. Rituximab has raised hopes
for a new therapeutic approach for patients with severe
vasculitic manifestations and active cryoglobulinaemic
nephropathy. Promising results have been reported
recently with a B-lymphocyte depletion protocol in
patients with active MC [13].

The present study focused on the effectiveness of
Rituximab on HCV-associated cryoglobulinaemic glo-
merulonephritis. Indications to treat included definite
bone marrow B-cell infiltration (two patients) and
resistance (two patients) or intolerance (two patients)
to conventional therapy. Patients invariably showed
a reduction in proteinuria, paralleled by a decrease in
the erythrocyte sedimentation rate, rheumatoid factor,
IgM levels and cryocrit, as well as a remarkable
increase in C4 levels with no substantial changes in
liver cytolytic enzyme levels. Moreover, no increase
in viral load was detected, even though incomplete
data limited evidence on this point. Possible long-term
implications of HCV RNA persistence is unpredict-
able at present. Association of Rituximab with effect-
tive antiviral agents might be a rational approach.
Normalization of bone marrow was also obtained
in the three cases examined before and after ther-
apy. Purpura disappeared in all patients. Arthralgia,
weakness and paraesthesia remarkably ameliorated.
As reported in a previous study [13], a decrease in IgM
levels was observed, while IgA levels were unaffected
and IgG was reduced only slightly. This is probably
due to the lack of CD20 expression on mature plasma
cells [20]. The selective depletion of IgM-producing
B cells represents a basis for treatment in cryoglo-
bulinaemic nephritis (Figure 2). Indeed, the IgM-κ
produced by a permanent clone of B cells promotes
the formation of IC with prolonged circulation in blood
[5] and shares strong affinity for the glomerular
matrix, which favours the deposition in the glomerulus
together with the IgG anti-HCV, previously bound
in circulation or subsequently fixed through an ‘in situ’
binding mechanism [4].

While the anecdotal and uncontrolled nature of
the study must be acknowledged, it should be
emphasized also that MC-associated nephritis repre-
sents a unique condition of an immune-mediated
disorder in which Rituximab specifically targets the
nephrotoxic Ig-producing cells and seems to be effec-
tive in the relatively long run. Nevertheless, further
large randomized controlled studies are needed to
confirm these encouraging results.

Conflict of interest statement. None declared.

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