CASE REPORT

Rituximab for the treatment of glomerulonephritis in hepatitis C associated cryoglobulinaemia

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ABSTRACT

Mixed-type cryoglobulins are strongly associated with hepatitis C virus (HCV) infection and may lead to vasculitis with renal involvement. The treatment of this condition is antiviral therapy for HCV, but this may be ineffective or not tolerated because of side effects. Alternative strategies such as immunosuppressive drugs and plasmapheresis are of limited use, especially in patients after liver transplantation (LTx). We describe an LTx patient with cryoglobulinaemia-associated glomerulonephritis, who was treated successfully with the B cell depleting monoclonal antibody rituximab.

KEYWORDS

Cryoglobulinaemia, glomerulonephritis, hepatitis C, liver transplantation, rituximab

INTRODUCTION

Cryoglobulins are plasma proteins that precipitate below 37°C, and consist of immunoglobulins with or without complement components.[1] Type 2 and 3 cryoglobulins are immunoglobulin G (IgG) complexed with monoclonal immunoglobulins (type 2), or polyclonal immunoglobulins (type 3). Usually, immunoglobulin M (IgM) is found as the complexing immunoglobulin. The type 2 and 3 cryoglobulins are together known as mixed-type cryoglobulins.

The immune complexes arise as a consequence of oligoclonal proliferation of B lymphocytes that produce autoantibodies against IgG.[2] The IgG binding property of these IgM antibodies is known as rheumatoid factor activity. An infection with hepatitis C virus (HCV) usually underlies the presence of mixed-type cryoglobulins and HCV antigens may be found within the circulating and precipitated immune complexes.[3,4] The immune complexes may precipitate within the glomeruli, thereby leading to glomerulonephritis with the typical histological appearance of membrano-proliferative glomerulonephritis (MPGN) type I.[4] This glomerulonephritis leads to progressive loss of renal function and an unfavourable prognosis for the survival of the patient.[5] The treatment of HCV-associated cryoglobulinaemia should be individualised and based on the severity of the symptoms. It consists of supportive care and antiviral or immunosuppressive therapy. Antiviral treatment may not be effective in clearing HCV and is sometimes poorly tolerated,[1] especially after LTx. Also, this strategy is of course not an option for the small group of patients with a non-HCV-related cryoglobulinaemia. To reduce the concentration of cryoglobulins and the related inflammation, high doses of corticosteroids with additional plasmapheresis or cyclophosphamide have been given. The efficacy of these treatment regimens is rather poor, with a risk of severe infections and an increase in HCV viral load.[6,7] Because of concomitant immunosuppressive treatment, the higher risk of serious infections and problems regarding graft rejection, the treatment options after LTx are limited even further. Alternative treatments with a greater efficacy and less toxicity are therefore needed. Rituximab may be such an alternative. Rituximab is a monoclonal antibody against the CD20 antigen on the cell surface of B lymphocytes. Recently, it has been reported that depletion of B lymphocytes by rituximab may lead to a complete remission of mixed-type cryoglobulinaemia-associated glomerulonephritis.[5] We describe a patient with mixed-type cryoglobulinaemia-associated MPGN type 1, who was successfully treated with rituximab, and review the current literature on this subject.

CASE REPORT

A 51-year-old male was admitted for clinical evaluation of oedema of the legs and progressive shortness of breath on physical exercise, which developed seven months after a

liver transplantation. His medical history showed a chronic hepatitis C (HVC genotype 1) viral infection, for which he had been treated with PEG interferon and ribavirin in the past, with relapse after the therapy was withdrawn. Finally, he developed decompensated liver cirrhosis and underwent orthoptic liver transplantation from a hepatitis B surface antigen-positive donor. The immunosuppressive medication consisted of cyclosporine and prednisone. In addition, he received lamivudin as antiviral medication to prevent hepatitis B reactivation. Six months postoperatively, routine liver biopsy showed recurrence of HCV infection with mild inflammation. He had documented type 2 diabetes mellitus, for which he used insulin subcutaneously. His renal function had previously been normal, without the presence of proteinuria.

At physical examination, obesity was noted with a weight of 125 km (height 190 cm), blood pressure 205/110 mmHg, heart rate 74 beats/min and an increased respiratory rate of 24/min. Remarkable findings included the presence of inspiratory crackles at the base of the lungs and pitting oedema at both lower extremities, extending to the knees. The examination of skin and joints was unremarkable.

The results of the laboratory examination of blood and urine are shown in *table 1*. Rituximab treatment was started two weeks after admission, during which time no significant change in renal function and proteinuria occurred. Hepatitis B DNA was not detectable in the serum by polymerase chain reaction. Ultrasonography showed kidneys of a normal size and appearance. A renal biopsy procedure was performed subsequently.

Microscopic examination of the renal biopsy showed 23 glomeruli in total, three of which were globally sclerosed. The intact glomeruli showed an increase in mesangial matrix and cells with splicing of the glomerular basement membrane. Microthrombi were observed in a few small vessels. In addition, diffuse tubulo-interstitial fibrosis with

tubulus atrophy was noted. By immunofluorescence, a strong granular pattern of IgM (3+), C3 (3+), IgA (2+), IgG (+), and C1Q (+) was noted, following the contours of the basal membrane. Electron microscopy revealed deposits within the basement membrane and subendothelially, confirming the light microscopic diagnosis of MPGN type 1.

Clinical course

The patient showed a severely compromised renal function with a nephrotic syndrome at presentation. The presence of cryoglobulins and high levels of serum IgM rheumatoid factor activity in a patient with chronic HCV infection made the diagnosis of mixed-type cryoglobulinaemia-associated glomerulonephritis most likely. This diagnosis was confirmed by renal biopsy. Despite maximal supportive therapy with diuretics, angiotensin-converting enzyme inhibiting medication and increasing the dose of corticosteroids, blood pressure and renal function did not improve and the clinical condition of the patient worsened. Therefore, treatment with anti-CD20 (rituximab) monoclonal antibody was started at a dose of 375 mg/m^2 at weekly intervals for four weeks. Corticosteroids were tapered to a dose of 2.5 mg daily. Two weeks after the first dose of rituximab the clinical symptoms improved, with a decrease in the proteinuria and serum cryoglobulin concentration. No side effects were noted during this treatment. Despite the disappearance of the clinical symptoms of the nephrotic syndrome, renal function did not improve within the first weeks. The tubulo-interstitial abnormalities suggested the possibility of cyclosporine nephrotoxicity. Therefore, this medication was substituted by mycophenolate mofetil. The outcomes of the laboratory tests are shown in *table 1*. During one year of follow-up, a significant improvement in renal function was observed with a decrease in proteinuria. The serum concentration of total IgM and IgM rheumatoid factor activity declined

Table 1. *Biochemical results after 3, 6 and 12 months after the first gift of rituximab*

	Starting rituximab	+3 months	+6 months	+12 months
Plasma creatinine (40-90 μmol/l)	221	139	154	133
Plasma albumin (35-45 g/l)	27	35	39	37
Plasma complement C3 (0.84-1.68 g/l)	0.72	1.20	1.15	1.13
Plasma complement C4 (0.16-0.42 g/l)	0.04	0.11	0.16	0.18
IgM rheumatoid factor activity (<12 IE/l)	800	100	25	25
Plasma cryoglobulins g/l	0.81	0.18	0.05	ND
Plasma IgG (7.0-16.0 g/l)	6.1	6.4	6,6	6.3
Plasma IgM (0.45-2.30 g/l)	3.0	1.95	0.83	1.04
B lymphocytes (x 10^9/l)	-	0	0	0
Protein in 24-hour urine (g)	11.7	3.72	4.14	3.51
Active urine sediment[*]	Yes	Yes	Yes	No
ALAT (0-40 U/l)	86	97	299	71
Viral load HCV (10^7 c/ml)	4.37	0.14	3.35	-

ND = not detectable. Reference values are between brackets. [*]Urine sediment containing >5 leucocytes or 2 erythrocytes per high power field (400 x).

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steeply within the first three months, while the total IgG concentration remained constant. After six months, cryoglobulins could no longer be detected and the levels of complement C3 and C4 had normalised. During the year of follow-up, no infections were seen and the HCV viral load decreased to the pre-existent level. However, after one year and with cryoglobulinaemia still in remission, active viral hepatitis was diagnosed by liver biopsy.

DISCUSSION

Mixed-type cryoglobulinaemia is highly associated with chronic HCV infection.[3] The circulating immune complexes may precipitate within the kidney, preferentially in the mesangium and in the subendothelial space. Splicing of the glomerular basement membrane is characteristic and caused by the ingrowth of mesangial cells. This can be visualised by light microscopy and is called the 'tram-track' phenomenon. These histological findings in the renal biopsy are characteristic for the diagnosis of membrano-proliferative glomerulonephritis (MPGN) type I. The presence of microthrombi in the small vessels, as was seen in our case, establishes the diagnosis of cryoglobulinaemia-associated MPGN with near certainty. MPGN type I is a rare diagnosis and the relation with a concomitant HCV infection is strong.[4,7] If not treated progressive renal failure will ensue. Glomerulonephritis is part of a spectrum of organ involvement in cryoglobulinaemic vasculitis, and other commonly observed manifestations are purpura, neuropathy, skin ulcera and arthralgias.[1]

Treatment options
The treatment of HCV-associated cryoglobulinaemia is based on elimination of HCV by the standard antiviral medication, PEG-interferon-alpha (IFN) combined with ribavirin.[1,6,8,9] Although effective (sustained viral response in 67 to 77% and a clinical response in 88%), this therapy is limited for several reasons. Despite effective antiviral treatment, cryoglobulinaemia may persist. Interferon may worsen or even induce symptoms of neuropathy, skin ulcers and renal insufficiency. With rapidly progressive or life-threatening disease the effect of antiviral treatment is too slow. Finally, IFN is contraindicated in advanced liver cirrhosis with risk of acute decompensation.[9] After LTx, antiviral therapy is less effective (sustained viral response 24 to 36%), poorly tolerated (severe haematological side effects in 36 to 60%) and may cause acute rejection of the graft in 5%.[10-14] If elimination of HCV is not possible, alternative strategies have been described, aiming at reducing the concentration of circulating immune complexes. The strategies include plasmapheresis and high-dose corticosteroids with or without cyclophosphamide. The effectiveness of these treatments is limited and potential side effects are severe

infections – especially in the already immunocompromised LTx patients – and increase of viral load with hepatocellular damage.[6,7]

In recent years the concept of anti-CD20 for mixed-type cryoglobulinaemia has emerged as an effective and safe treatment, inducing a rapid remission of disease activity.[15,16] In the groundbreaking studies by Zaja *et al.* and Sansonno *et al.*, two series of patients with mixed-type cryoglobulinaemia were treated with rituximab. Patients in the study by Sansonno *et al.* had a follow-up of 12 months and 80% showed a complete remission without recurrence of disease. Only one of the 20 patients had nephritis that did not respond to treatment. In the study by Zaja *et al.* 15 patients were treated with rituximab with a follow-up of 9 to 31 months. Within the study, two patients were present with renal involvement, of which one did not respond to therapy and the other patient showed a complete remission. These studies were followed by two reports[5,17] of a series of patients with cryoglobulin-associated nephritis who were treated successfully with anti-CD20 monoclonal antibody infusion, and a number of case reports thereafter.[18-21] In the study by Rocatello *et al.*,[17] all five patients with cryoglobulin-associated nephritis showed a partial or complete response after rituximab treatment. In the follow-up period of 12 to 18 months no recurrence was noted. In the study by Quartuccio *et al.* also five out of five patients with cryoglobulin-associated nephritis responded to rituximab treatment but at follow-up (9 to 21 months), three out of five patients showed a recurrence of disease at five, seven and 12 months. A repeated cycle of rituximab infusion again induced remission of disease activity.[5] Of note is that in all studies the initial treatment schedule is based on the original non-Hodgkin's lymphoma study and consists of four weekly infusions of rituximab 375 mg/m².[22] It is not known whether lower or less frequent dosing may be equally effective.

Mechanism of action
Rituximab is an effective depleting monoclonal antibody for circulating CD20 bearing B lymphocytes, although in the secondary lymphoid organs a large number of the B lymphocytes remain present. Months (on average 6 to 9 months) after the last infusion, the B lymphocytes reappear in the circulation.[5,15,16] The IgM autoantibody producing B lymphocytes appears to be depleted preferentially, which may explain the efficacy of rituximab in the treatment of mixed-type cryoglobulinaemia and rheumatoid arthritis.[5] Also in our patient there was a clear decrease in the concentration of IgM rheumatoid factor activity, which correlated with the decrease of total serum IgM and cryoglobulin concentration. The observed improvement of creatinine clearance may in part be related to the withdrawal of cyclosporine in this specific case. However, the decrease in proteinuria and disappearance of an active urine sediment suggests the resolution of ongoing nephritis, by

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limiting the deposition of newly formed cryoglobulins. The persistence of proteinuria in our patient, although reduced by 70% one year after rituximab treatment, may indicate permanent damage to the glomeruli.

Side effects of rituximab

Expression of the CD20 antigen is limited to immature and mature B lymphocytes. The long-lived plasma cells do not carry the CD20 antigen on their cell surface and are therefore not deleted. This probably explains why serum IgG concentration remains unchanged after anti-CD20 therapy. This leaves the humoral immunity intact and there is no necessity for prophylactic infusion with immunoglobulins. However, infections with human parvovirus B19 and reactivation of a hepatitis B viral infection have been described in association with rituximab.[23,24] The frequency of infectious complications seems low. Possibly, the risk for infectious complications is increased in already immunocompromised patients, such as patients taking immunosuppressive drugs (for instance transplant patients) or suffering from an HIV infection. Serious infectious complications have been reported in renal transplant patients receiving rituximab. However, these data are anecdotal.[25,26]

Patients with chronic HCV have received rituximab without an increase in HCV disease activity.[5,15,16] Of course, it cannot be excluded that the increase in HCV hepatitis disease activity in our patient, who was also taking immunosuppressive medication, was related to the rituximab treatment. The immediate side effects after infusion of rituximab are usually few and mild.[5,15-17] However, the side effects of anti-CD20 therapy in the long term, especially in relation to the function of the immune system, are not known.

CONCLUSION

Cryoglobulinaemia is a current clinical problem in HCV-infected patients, even after liver transplantation for HCV cirrhosis. Depletion of B lymphocytes by infusion of the monoclonal antibody rituximab appears to be a rapid, effective and non-toxic treatment for mixed cryoglobulinaemia-associated glomerulonephritis. Rituximab has emerged as an important new drug for the treatment of this condition, especially in selective cases in which the current therapeutic options are limited.

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