Prolonged disease-free remission following rituximab and low-dose cyclophosphamide therapy for renal ANCA-associated vasculitis


Renal Section, Department of Medicine, Imperial College London, UK, West London Kidney and Transplant Centre, Hammersmith Hospital, London, UK and UCL Centre for Nephrology, Royal Free Hospital, London, UK

Correspondence and offprint requests to: Alan D. Salama; E-mail: a.salama@medsch.ucl.ac.uk

Abstract

Background. Rituximab (RTX) has been shown to be effective as an induction agent in anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV), but studies have been limited by short-term follow-up. We decided to investigate the long-term efficacy and safety of an RTX-based cyclophosphamide (CYP)-sparing regimen (CycLow-Vas) for renal AAV.
ANCA vasculitis treatment

Methods. Consecutive patients with renal AAV presenting de novo or with a major relapse, except those with serum creatinine >500 μmol/L, previous treatment with RTX and pulmonary haemorrhage or cerebral vasculitis, were treated with two pulses of RTX 2 weeks apart and six fortnightly doses of CYP, as well as a reducing protocol of daily oral steroids. Maintenance was with low-dose steroids and azathioprine.

Results. Twenty-three patients were treated. Median follow-up was 39 months, with 17 patients reaching >2 years of follow-up. All patients achieved clinical remission within 6 weeks. Three major and two minor relapses occurred in five patients at a median of 30 months, which were treated by re-dosing with RTX for major relapses and steroid increase alone for minor relapses. Adverse events included one severe drug reaction, four non-serious and one serious infective episodes in the first 3 months, one skin malignancy at 21 months and one death at 19 months not related to treatment or disease.

Conclusions. A RTX-based low-dose CYP regimen is effective at inducing long-term disease-free remission and may be the platform on which to develop a steroid-minimizing regimen to further decrease adverse events in the future.

Keywords: ANCA; efficacy; glomerulonephritis; rituximab; vasculitis

Introduction

The anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitides (AAV) are multisystem autoimmune conditions characterized by the presence of ANCA and small vessel inflammation. They include Wegener’s granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome. Although the factors determining the extent of organ involvement are unknown, the kidney is a common site of disease. Prior to the routine use of immunosuppression, the prognosis of renal vasculitis was poor, with 80% of patients dying within 1 year [1]. The use of immunosuppressive therapy dramatically improved survival but resulted in significant morbidity, especially in the early phase of treatment [2]. Those at highest risk of therapy-induced complications include the elderly and those with more advanced renal disease [3]. In addition, since these conditions have a tendency to relapse, patients are exposed to repeated cycles of therapy, which increases the risk of drug-related adverse events.

Current standard therapy comprises two separate phases—high-dose induction and low-dose maintenance—to enable rapid disease remission and long-term disease-free survival [4]. Standard induction therapy consists of high-dose corticosteroids and cyclophosphamide (CYP), either as continuous oral or pulsed intravenous (IV) therapy, for 3–6 months [5]. Maintenance therapy replaces CYP with azathioprine or methotrexate and allows for variable steroid tapering [4]. The duration of immunosuppression is not standard and current trials are seeking to investigate the ideal duration of maintenance therapy. However, it is generally accepted that patients with proteinase-3-specific ANCA (PR3-ANCA) are at greater risk of relapse than those with myeloperoxidase-specific ANCA (MPO-ANCA) and are therefore kept on maintenance therapy for longer [6]. CYP use is associated with multiple adverse drug reactions, many of which are dose dependent. These include cytopenias, infections, haemorrhagic cystitis, myelodysplasia, infertility and bladder, skin and haematological malignancies [7–9]. Previous studies have demonstrated that using pulsed CYP rather than daily oral therapy is as effective in inducing remission and is associated with fewer leucopenia episodes [5].

Rituximab (RTX) is a chimeric antibody directed against human CD20, expressed on immature and mature B cells. Preliminary studies have demonstrated its effectiveness in relapsing or refractory ANCA-associated vasculitis [10–13]. In addition, short-term results of two controlled trials of RTX for induction therapy in AAV have recently been reported [14, 15], demonstrating equivalence with conventional CYP regimens in the short term.

In January 2006, a treatment protocol was adopted at West London Renal and Transplant Centre (WLRTC) for the management of renal AAV. The protocol involved the use of RTX as a CYP sparing agent, enabling a cumulative dose of no >3.5 g of CYP per patient episode. We have followed this cohort for a median time of over 3 years and now report the efficacy and safety of this regimen in providing prolonged disease-free remission.

Materials and methods

This was a single centre prospective cohort study. All patients referred to the WLRTC with a diagnosis of AAV and renal involvement were considered for inclusion in this protocol. Inclusion criteria were AAV and active renal disease as defined by the presence of circulating ANCA, an active urinary sediment and histological evidence of pauci-immune necrotizing glomerulonephritis on renal biopsy. Some patients with previously documented disease were included based on a clinical diagnosis of disease relapse, with development of active urinary sediment and declining renal function in the presence of rising ANCA titres. Patients were excluded if they presented with a serum creatinine >500 μmol/L (5.7 mg/dL), evidence of pulmonary haemorrhage or cerebral vasculitis and were treated with plasma exchange and a standard CYP-based regimen published previously [16].

The treatment protocol (Figure 1) consisted of RTX 1 g at Day 0 and 14. Intravenous CYP was given at Day 0 and then every 14 days for a total of six doses. The first two doses were 10 mg/kg, with a maximum dose of 750 mg, the final four doses were 10 mg/kg to a maximum of 500 mg. Oral prednisolone was started at Day 0 at a dose of 1 mg/kg with a maximum dose of 60 mg. This was reduced sequentially to achieve a dose of 10 mg by Week 13. Further steroid weaning was then postponed until 1 year. Any patient who had received pulsed IV methylprednisolone at a referring hospital was started at 30 mg prednisolone for the first 3 weeks. Azathioprine was commenced at 3 months, at a dose of 2 mg/kg (maximum 150 mg) if thalidimide methyltransferase activity was normal or 1 mg/kg if low.

All patients received prophylactic co-trimoxazole for 3 months, proton pump inhibitors and bone protection with calcium/D3, while patients originating from high-risk regions were also given prophylaxis against tuberculosis with isoniazid and pyridoxine.

All patients were scored for disease activity using version 3 of the Birmingham Vasculitis Activity Score (BVAS) [17]. Remission was based on clinical and biochemical criteria as well as a BVAS of zero or BVAS ≤ 5 if all scores were due to persistent haematuria or proteinuria in the presence of stable or improving renal function as measured by serum creatinine. Estimated glomerular filtration rate (eGFR) was measured using the four variable Modification of Diet in Renal Disease calculation [18]. Proteinuria was estimated by spot urine protein: creatinine ratios. All patients were ANCA positive on immunofluorescence, and titres of either anti-proteinase-3 (PR3) or anti-myeloperoxidase (MPO) antibodies were sequentially measured using luminex technology (BMD FIDIS luminex, Technoclone, UK).
Subjects

Twenty-three consecutive patients were treated. Five potentially eligible patients during this period were not treated with this protocol—two as they were enrolled in other multicentre trials and three due to clinical exclusion. The baseline characteristics are summarized in Table 1. All patients who reached at least 12 months of follow-up have been included for analysis. Seventeen patients reached at least 24 months of follow-up. Twenty-two of the 23 patients had a new diagnosis of AAV, and all but one, treated for Waldenstrom’s macroglobulinaemia, had not received prior immunosuppressive therapy. The patient with relapsing disease was taking mycophenolate mofetil at the time of relapse. This patient received only two doses of CYP and at 6 weeks was switched back to pre-relapse immunosuppressive. Two patients did not complete the induction protocol—one due to a serious unexpected adverse event, the second due to gastrointestinal side effects following each of the doses of CYP. Both have been included in the analysis. Both patients received only two of the CYP doses but both RTX doses. All patients had generalized disease, with impaired kidney function and 91% had a confirmatory renal biopsy showing focal necrotizing glomerulonephritis. The median cumulative dose of CYP was 3.44 g (range 1–3.5). Seven of the 23 patients were of Indo-Asian origin and their average age was significantly lower than in the non-Indo-Asian patients (49.7 versus 64.8 years, respectively; P = 0.009, Mann–Whitney U-test) in keeping with a changing ethnic demographic that we have previously noted in London.

Disease severity, as assessed by BVAS, demonstrated that most patients had significant extrarenal disease in addition to their renal involvement. Median BVAS at entry was 21 (range 12–27).
Disease remission

All but one patient achieved clinical remission within 6 weeks of presentation; the exception was the patient who suffered a major adverse event making it difficult to assess disease activity. At 6 weeks, 21 of the 23 patients had a BVAS of greater than zero only due to persistent urinary abnormalities but had renal function that had stabilized or improved as assessed by declining serum creatinine. By 6 months, all the patients had a BVAS of zero (Figure 2). All patients except one with a reaction to the second dose of RTX had C-reactive protein within the normal range (<10 mg/L) at 4 weeks (Figure 3). The median anti-PR3/MPO ANCA titre fell from 310 IU/mL (range 61–1697) to 28 IU/mL (range 1–137) by 3 months (NR < 25 IU/mL) (Figure 4).

Median eGFR increased from 24 mL/min (range 8–63) at presentation to 33 mL/min (10–66) at 1 month and 42 mL/min (8–81) at 6 months (Figure 5). One patient presented with an eGFR of 8 mL/min and although she went into remission clinically, her eGFR never improved to >11 mL/min and she commenced dialysis 8 months later.

B cells

All patients showed depletion of circulating CD19-positive B cells (depletion defined as absolute number of B cells < 10 cells/µL). Fourteen patients reconstituted their circulating B cells within the follow-up period. The median time to reconstitute CD19 + cells in these 14 patients was 97 weeks (range 32–156) (Figure 6). Of these 14 cases, 9 remained in remission for a median of 49 weeks (range 0–137) since their first recorded B-cell reconstitution. Three patients had major relapses at 0, 16 and 38 weeks following B-cell reconstitution. One minor relapse occurred at the time of B-cell reconstitution, and one occurred 4 weeks following B cell return. Nine patients remained B-cell deplete for a median of 78 weeks (range
27–130). Only one patient had a prolonged mild hypogammaglobulinaemia during the study (Serum IgG 4.8 g/L; normal range 5.4–16 g/L), while four others had transient hypogammaglobulinaemia, which normalized by 6 months.

Relapse

Three patients had renal relapses—one at 17 months and two at 3 years. All three patients had reconstituted their B cells at the time of relapse (Figure 6). Two were PR3-ANCA positive, one MPO-ANCA. All three relapses were preceded by a significant rise in their PR3/MPO-ANCA titre (mean pre-relapse 8 IU/mL, mean at relapse 304 IU/mL). All three had repeat renal biopsies demonstrating focal necrotizing glomerulonephritis, while eGFR had declined by 13–35% at the time of relapse. Two were retreated with the full protocol and one with only RTX and steroids. All three achieved clinical remission, with two of the three returning to their pre-relapse renal function and the third having not fully recovered at 5 months (pre-relapse eGFR 59, worst 38, most recent 45 mL/min). All three renal relapses were associated with relapse of extra-renal symptoms. In addition, two patients had minor relapses with arthralgias. The first, at 22 months, had repopulated her B cells and had a significant rise in her PR3-ANCA titres. The second, at 30 months, had not reconstituted his B cells and had no significant rise in PR3-ANCA titres. Both responded rapidly to a short course of oral corticosteroids.

One patient had Waldenström’s macroglobulinaemia in addition to her ANCA-associated biopsy proven pauci-immune glomerulonephritis. She responded well to initial therapy but at 42 months developed a relapse of her Waldenström’s, with a paraprotein of 27 g/L, and was treated by her oncologists with RTX. The PR3-ANCA titres were raised with this episode, as was the serum creatinine. She was not re-biopsied but responded to therapy with a reduction in her paraprotein and a return to baseline renal function.

Major adverse events

One elderly patient, with concurrent pulmonary fibrosis and cardiac failure, developed a severe systemic illness 2 days following his second dose of RTX and CYP, characterized by hypotension and multi-organ dysfunction, which necessitated support on the intensive therapy unit (Table 2). He developed acute kidney injury and required temporary continuous veno-venous haemofiltration. His clinical condition rapidly improved, as did his renal function, to pre-treatment levels. As a result, he did not complete the immunosuppressive protocol and only received two doses of RTX and CYP. All bacteriological tests were negative; hence it is unclear whether this was a septic episode or a ‘cytokine storm’-like response to the immunosuppressive therapy. This patient died 19 months following presentation, after a prolonged hospital admission with decompensated cardiac failure, chest infection and a fractured neck of femur. He had been maintained from 5 months on 10-mg/day prednisolone monotherapy due to leucopenia with azathioprine. His renal function had remained stable throughout his final admission, and his MPO-ANCA titre remained unchanged within the normal range.

Minor adverse events

There were six documented minor infections (five urinary tract, one lower respiratory tract) during the first 3 months that responded to oral antibiotics. There were four documented minor infections (two urinary tract, two lower respiratory tract) between 3 and 6 months. One patient developed shingles at 2 years, and two patients required...
brief hospitalization for bacteriology—negative gastrointestinal at 12 and 26 weeks (Table 2).

**Immunosuppression-related events**

No patients developed leucopenia during the first 3 months (Figure 7). Three patients had curtailed CYP treatment, as described above. None of the cohort developed any severe sequelae of CYP therapy. Three patients developed leucopenia while on azathioprine. Two of these responded to dose reductions, while one was unable to tolerate further immunosuppressive medication and was left on oral corticosteroids alone. One patient developed a hypersensitivity reaction, one an isolated skin rash and one gastrointestinal disturbance. These patients were all converted to mycophenolate mofetil. One patient had a squamo-proliferative lesion removed from his scalp at 21 months.

**Discussion**

This study reports a large single-centre series of patients with renal AAV treated with RTX and low-dose CYP as induction therapy, attempting to maximize the benefit of these agents while minimizing toxicity, in a manner analogous to the use of combined chemotherapy for the treatment of certain cancers. Previously reported studies have documented the efficacy of higher dose CYP, either as continuous oral or pulsed intravenous therapy, in combination with oral corticosteroids as induction therapy [4, 5]. The CYCLOPS study compared induction with pulsed or daily oral CYP in AAV, with patients who had serum creatinine $<$ 500 μg/L. The cumulative doses of CYP used in that study were considerably higher than in our protocol (median cumulative dose 8.2 g in pulsed intravenous and 15.9 g in continuous oral CYP arms compared with 3.4 g in our study) and were associated with significant serious adverse events [5]. The absence of any significant leucopenia episodes and the low incidence of infective episodes in our study compared favourably to the CYCLOPS study, in which 26% of intravenous and 45% of oral CYP-treated patients suffered leucopenia and in which 22 and 29 infective episodes occurred into the two groups, respectively. This demonstrates that a significantly reduced dosing regimen of CYP is less toxic in the short term. Moreover, since recent studies have demonstrated the close relationship between the total cumulative dose of CYP and long-term malignancy risk [7], manoeuvres which reduce the total CYP exposure are to be welcomed.

While we achieved disease remission in all our patients, other trials have reported lower rates of remission. In part, this relates to definitions of remission used. In our study, we reported remission once patients had resolution of their presenting features with biochemical evidence of normalization of acute inflammatory responses and no new or worsening symptoms on BVAS scoring. However, more recent trials have reported remission as being disease free off all medication [15] or being in sustained remission (for 6 months) at trial termination [14].

Recent randomized trials have demonstrated that CYP may be reduced or omitted as part of induction therapy. These trials have reported only short-term follow-up (12 months in RITUXVAS and 6 months in RAVE). The impact of CYP reduction on disease relapse and durability of remission is therefore still unknown. Our data suggest that such an approach results in prolonged disease-free remission, with only one major (renal) and two minor relapses by 18 months, representing a 13% relapse rate. This is comparable to data from the CYCAZAREM trial [4], which compared maintenance of disease remission between azathioprine and CYP, following CYP induction therapy. One important difference between our cohort and the two RTX trials is the routine use of azathioprine maintenance in RTX-treated patients. Since our population consisted mostly of PR3-ANCA patients (70%), they may have benefited from prolonged immunosuppression to maintain remission, unlike MPO-ANCA patients in whom immunosuppression may be safely reduced or withdrawn after remission induction [19].

Disappointingly, despite CYP reduction/exclusion, neither the RAVE nor RITUXVAS studies demonstrated a significant reduction in serious adverse events, suggesting that some of the adverse events may relate to the large steroid dose that was employed. Our cohort had 1/23 patients who suffered a serious adverse event, 2 days following RTX dosing, making it likely that the drug was contributory. However, the small number of other (mainly minor infective) adverse events in a population with a mean age of 60.2 years and mean eGFR of 26.1 mL/min is reassuring. Other EUVAS studies have demonstrated that adverse events are related to creatinine at entry and age [2]. In the CYCAZAREM study, the mean age was comparable to our cohort at 58 years, while eGFR was significantly higher at 49.2 mL/min, and the authors reported 10% serious adverse events [4]. In contrast, in RITUXVAS, the median age was 67 years and entry eGFR was 18 mL/min. In this study, adverse events were reported in 41% of patients [14].

Apart from the relatively long follow-up period, our cohort differed from both the RAVE and RITUXVAS groups in disease burden and ethnicity. RITUXVAS included patients with more severe disease involvement, with dialysis dependency, pulmonary haemorrhage and CNS vasculitis. In RAVE, 92% of patients were Caucasian, and while all patients with ANCA-associated disease were included, only 66% had renal involvement and of these, none had a serum creatinine $>$ 4 mg/dL (354 μmol/L). Our cohort falls in between these two groups, with 30% Indo-Asian ethnicity and significant renal disease but no ‘life threatening’ complications. Our study was not a controlled trial and therefore the results were not tested against standard therapy; however, we found that this low-dose CYP and RTX-based induction, followed by azathioprine maintenance, provided an efficacious regimen with an acceptable safety profile. This should be tested formally in a randomized clinical trial against standard therapy. Additionally, we believe that such a regimen could be the platform from which to attempt steroid minimization in this highly susceptible elderly population with renal impairment. This may be of particular importance in our patient population since our cohort included 30% Indo-Asian patients, in whom there is known to be excess long-term sequelae from corticosteroids [20].

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References


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