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Clinical outcome in patients with chronic antibody-mediated rejection treated with and without rituximab and intravenous immunoglobulin combination therapy

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ABSTRACT

We previously reported that rituximab (RTX) and intravenous immunoglobulin (IVIg) combination therapy (RIT) is effective in treating patients with chronic active antibody-mediated rejection (CAMR), and the proteinuria level can determine the response to RIT. However, the results were not compared to those of patients who did not receive RIT. Fifty-nine patients with CAMR were divided into 2 groups: an RIT treated group ($n = 25$) and a historic control (HC) group who had not received RIT ($n = 29$). The RIT group was treated with RTX (375 mg/m^2) and IVIg (0.4 g/kg) for 4 days. We compared the decline in glomerular filtration rate/month (ΔeGFR), RIT-related complications, and allograft survival rate in both groups. We also compared the allograft survival rate between patients with high proteinuria (spot urine protein/creatinine [PC] ratio >3.5 g/g) and low proteinuria (PC ratio <3.5 g/g). ΔeGFR was significantly decreased in the RIT group compared with the HC group after 6 months ($P < 0.05$). No serious complications were associated with RIT, and only one case of herpes zoster infection developed. The overall allograft survival rate in the RIT group was significantly higher than in the HC group. In both groups, patients with low proteinuria survived better than patients with heavy proteinuria ($P < 0.05$). The allograft survival rate was greater in the high proteinuria RIT group than that in the HC group. RIT treatment is recommended for delaying the progression of CAMR without serious complications, and is not limited by the presence of heavy proteinuria.

1. Introduction

Chronic antibody-mediated rejection (CAMR), which was included as a new disease entity in the update of the Banff 05 classification, is one of the main causes of late allograft loss [1,2]. This disease has received increasing attention because of its poor prognosis — even the most recent T cell targeting immunosuppressants cannot prevent or reverse it [2–4]. Some researchers have proposed that therapies directed at the humoral immune response may be required to successfully treat CAMR, since antibody-mediated tissue injury, rather than T-cell-mediated immunity, is associated with its development [5–7].

During recent years, combined use of rituximab (RTX) and intravenous immunoglobulin (IVIg) therapy has been trialed in patients with CAMR [7–10]. Improved allograft function was observed after treatment with RIT in pediatric and adult patients with CAMR [8–10]. Our previous reports also demonstrated that the combined use of RTX and IVIg effectively delayed CAMR progression, and the amount of proteinuria at diagnosis of CAMR was a prognostic factor for the response to therapy [11,12].

Despite the proven effect of RIT in delaying the progression of CAMR, it is uncertain whether it promotes renal allograft survival, because most previous studies were single-arm studies, which only investigated the change in clinical parameters that indicate allograft function before and after treatment. To address this, we assessed the clinical data relating to allograft function of patients who were previously diagnosed with CAMR, but who had not taken RIT. We designated this group of patients as the historic control group (HC). By comparing the clinical outcomes of patients who took RIT with those of patients who did not, we intended to investigate the clinical usefulness of RIT in promoting allograft survival after the diagnosis of CAMR.

Abbreviations: CAMR, chronic antibody mediated rejection; eGFR, estimated glomerular filtration rate; HC, historic control; HP, high proteinuria; IVIg, intravenous immunoglobulin; LP, low proteinuria; PC ratio, spot urine protein to creatinine ratio; RIT, rituximab–intravenous immunoglobulin combination therapy; RTX, rituximab.

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2. Patients and method

2.1. Study population and diagnosis of CAMR

We included patients who were diagnosed with CAMR by allograft biopsy between January 2001 and December 2013 in Seoul St. Mary's hospital. The diagnosis of CAMR was based on the updated Banff classification, as described in previous reports [1,12]. Briefly, (1) transplant glomerulopathy and severe peritubular capillary basement membrane multi-layering (PTCBMM), interstitial fibrosis (IF), and tubular atrophy (TA), with or without peritubular capillary (PTC) loss, and fibrous intimal thickening in arteries without internal elastica duplication; (2) diffuse C4d deposition in PTCs; and (3) presence of donor-specific anti-HLA antibody (DSA). This study was approved by the institutional review board of our institution (KC12RISI0070).

2.2. Clinical and biochemical data

We collected the baseline characteristics of the patients including sex, age, cause of end stage renal disease (ESRD), dialysis type, and duration before kidney transplant (KT). Clinical data about the transplantation included the number of transplants, number of HLA mismatches, donor type, and main immunosuppressive medication.

2.3. Protocol of rituximab/IVIg combination therapy for CAMR

In 25 patients, we used combination therapy composed of RTX and IVIg. The protocol has been described previously (RIT protocol) [11,12]. Briefly, RTX (375 mg/m^2) was infused on day 1, followed by IVIg (0.4 g/kg) once daily for 4 days. Pulse methylprednisolone at a dose of 500 mg IV was administered daily for the first 3 days, followed by oral prednisolone, tapered to 30 mg/day. We measured anti-HLA antibody using Luminex solid-phase assays (LSA; Tepnel Lifecodes Corp., Stamford, CT) at the time of biopsy as described previously [13]. Where anti-HLA antibodies detected in the patient corresponded to the HLA type of the donor, these were regarded as donor-specific anti-HLA antibodies (HLA-DSA). The results were presented as 4 levels, according to the median fluorescent intensity (MFI) value: strong, >10,000; moderate, 5000–10,000; weak, 1000–5000; and negative, <1000. In the HC group, steroid pulse therapy was used in all patients, with administration of 125 mg methylprednisolone twice daily for 3 days, followed by 30 mg oral prednisolone.

2.4. Efficacy of treatment protocol

The primary outcome of this study was the change in allograft function, and the secondary outcome was allograft survival rate after the diagnosis of CAMR. We assessed allograft function on the basis of serum creatinine levels and estimated glomerular filtration rate (eGFR), using the modification of the diet in renal disease (MDRD) formula (eGFR = $175 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.263}$ [$\times 0.742$ if female] mL min^{-1} · 1.73 m^{-2}) at 6-month intervals until the last follow-up [14]. In addition, we calculated the decline in eGFR rate per month (ΔeGFR) during the 6 months before and after RIT (or before and after biopsy in the HC group). We compared eGFR at each time point (−6 months, at biopsy, +6, +12 months from RIT or biopsy, and last visit) between the RIT and HC groups, and compared ΔeGFR during the 6 months before and after the diagnosis of CAMR and at 6-months intervals, until the last follow-up.

In our previous report, the amount of proteinuria was the most important factor in predicting the response to RIT [11]. We performed ROC analysis in 25 RIT group patients to assess the optimal level of proteinuria required for predicting the response to RIT. Afterwards, we investigated whether the amount of proteinuria was associated with the allograft survival rate in the RIT and HC groups, respectively.

2.5. Statistical analysis

Statistical analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD or counts and percentages, depending on the data type. For continuous variables, means were compared using Student's *t*-test. For categorized variables, Pearson's chi-square test and Fisher's exact test were used. Receiver operating curve (ROC) analysis was used to investigate the prediction of the response to RIT therapy. The changes in eGFR before and after treatment within the same group were evaluated by paired comparison. Graft survival rates were calculated using Kaplan–Meier analysis, and we used the log–rank method to compare survival rates between the RIT and HC groups. Binary logistic regression analysis was used to investigate whether RIT therapy improves allograft outcome independently. All tests were two-tailed, and the results were considered significant when the *P* value was below 0.05.

3. Results

3.1. Comparison of baseline characteristics

Patient characteristics in the RIT and HC groups are shown in Table 1. The mean age of the patients at the time of CAMR diagnosis, sex ratio, primary renal disease, dialysis type before KT, and dialysis duration did not differ significantly between both groups ($P > 0.05$ respectively). Clinical characteristics and laboratory findings at biopsy such as the length of time from KT to the diagnosis of CAMR, serum creatinine, MDRD eGFR, and the amount of proteinuria at biopsy also did not differ between two groups.

3.2. Comparison of the change of allograft function before and after the diagnosis of CAMR

In the RIT group, all patients tolerated medication well and completed their treatment without immediate adverse effects. Fig. 1A presents the change of eGFR before and after the diagnosis of CAMR. Before biopsy, a progressive decrease of eGFR was found in all patients from both groups. At 6 months before biopsy, the average eGFR was 44.5 ± 17.4 mL min^{-1} · 1.73 m^{-2} and 40.1 ± 15.3 mL min^{-1} · 1.73 m^{-2} in the RIT and HC groups, respectively. They progressively declined to 34.4 ± 14.0 mL min^{-1} · 1.73 m^{-2} and 34.2 ± 14.7 mL min^{-1} · 1.73 m^{-2} at the time of biopsy in RIT and HC group, respectively ($P < 0.001$ at each comparison). The calculated ΔeGFR was 1.3 ± 1.3 mL min^{-1} · 1.73 m^{-2} per month in the RIT group, and it was 1.0 ± 1.8 mL min^{-1} · 1.73 m^{-2} per month in the HC group during that period. eGFR at 6 months before the diagnosis of CAMR, at the time of diagnosis of CAMR, and the calculated ΔeGFR did not differ between two groups ($P = 0.44$, $P = 0.97$, and $P = 0.56$

Table 1
Comparison of baseline and clinical characteristics of patient populations.

Clinical parameters	RIT group ($n = 25$)	HC group ($n = 29$)	*P*
Age (years)	44.0 ± 7.1	49.5 ± 10.1	0.12
Male gender, *n* (%)	18 (72.0)	19 (65.5)	
Primary renal disease			
cGN, *n* (%)	12 (48)	19 (65.5)	
HTN, *n* (%)	7 (28)	3 (10.3)	0.26
DM, *n* (%)	1 (4)	3 (10.3)	
Unknown, *n* (%)	5 (20)	4 (13.8)	
Dialysis type before KT	19.4 22.7	21.3 18.5	0.75
Hemodialysis, *n* (%)	17 (68)	22 (75.9)	
Peritoneal dialysis, *n* (%)	7 (28)	7 (24.1)	0.51
Preemptive, *n* (%)	1 (4)	0 (0)	
Dialysis duration, month	19.4 ± 22.7	21.3 ± 18.5	0.75
Donor type, living, *n* (%)	19 (76)	25 (86.2)	0.49
Re-transplantation, *n* (%)	2 (8)	5 (17.2)	0.43
Main immunosuppresant			
Cyclosprine, *n* (%)	8 (32)	25 (86)	<0.01
Tacrolimus, *n* (%)	17 (68)	4 (14)	
HLA mismatch number	3.5 ± 1.5	3.6 ± 1.2	0.74
Time between KT and biopsy, month	92.7 ± 66.0	113.6 ± 59.4	0.23
Serum creatinine (mg/dL) at biopsy	2.4 ± 1.0	2.6 ± 0.9	0.47
MDRD eGFR (mL/min/1.73 m^2) at biopsy	44.5 ± 17.4	41.0 ± 15.4	0.63
Proteinuria (g/day) at biopsy	3.1 ± 3.4	4.4 ± 5.7	0.30

CAMR, chronic antibody mediate rejection; cGN, chronic glomerulonephritis; HTN, hypertension; DM, diabetes mellitus; MDRD eGFR, estimated GFR using the Modification of Diet in Renal Disease Study equation, KT; kidney transplantation.