Effect of Rabbit Antithymocyte Globulin on Acute and Chronic Active Antibody-Mediated Rejection After Kidney Transplantation

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ABSTRACT

Background. Rabbit antithymocyte globulin (rATG) induction is associated with reduction in the occurrence of de novo donor-specific antibody (DSA) and antibody-mediated rejection (AMR). Therefore, rATG administration is considered as a treatment for AMR. However, only a few studies have investigated the treatment of AMR with rATG after kidney transplantation.

Methods. Between April 2013 and March 2018, 162 consecutive de novo kidney transplantations were performed with induction immunosuppressive therapy comprising tacrolimus, mycophenolate mofetil, methylprednisolone, and basiliximab. AMR was diagnosed on the basis of the presence of DSA and episode biopsy findings. For DSA-positive recipients, plasmapheresis was performed to remove DSA before rATG administration (1.5 mg/kg for 5 days). Patients treated with rATG against active AMR were retrospectively analyzed for graft function.

Results. A total of 13 kidney transplant recipients developed active AMR within 302 days after transplantation. After rATG administration, the mean serum creatinine and urine protein levels significantly declined from 3.03 mg/dL to 1.68 mg/dL ($P = .002$) within 46 days and from 3.01 g/gCr to 0.54 g/gCr ($P = .006$) within 106 days, respectively. The peripheral blood lymphocyte count rapidly decreased after rATG administration and remained low for 12 months. With regard to adverse events, fever (84.6%), cytomegalovirus viremia (84.6%), thrombocytopenia (61.5%), anemia (30.8%), and neutropenia (15.4%) occurred within 3 months after rATG administration.

Conclusions. rATG improved graft function by suppressing peripheral blood lymphocytes in kidney transplant recipients with active AMR. The rATG administration as a treatment for active AMR may contribute to positive graft outcomes after kidney transplantation.

ANTIBODY-MEDIATED rejection (AMR) is the most common cause of graft failure after kidney transplantation despite the significant advances in immunosuppressive therapy [1–3]. Active AMR is difficult to treat because it is typically less responsive to conventional treatment regimens, such as plasmapheresis, intravenous immunoglobulin, and rituximab. A systematic review of the treatment of acute AMR in kidney transplant recipients concluded that data describing the efficacy of treatments for AMR in renal allografts are of low or very low quality [4]. The most important strategy for the treatment of AMR is to remove existing donor-specific antibodies (DSAs) and eradicate the clonal population of B cells or plasma cells responsible for their production.

Rabbit antithymocyte globulin (rATG) has been widely used as an anti-T cell agent for the treatment of acute T cell-mediated rejection in kidney transplant recipients.

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rATG is a polyclonal antibody that targets various T cell surface antigens as well as contains antibodies against natural killer cell antigens, B cell antigens, plasma cell antigens, adhesion molecules, and chemokine receptors [5]. As a result, rATG displays a mechanism for the depletion of a variety of immune cells [6]. Observational data suggest that rATG induction inhibits DSA production, with a potential role in either reducing the risk of AMR with high-strength preformed DSA or lowering de novo DSA in moderately sensitized patients [7,8]. Therefore, rATG administration is expected to treat AMR after kidney transplantation.

However, rATG is rarely used practically for the treatment of AMR in kidney transplant recipients [9]. Only a few studies have examined the treatment of AMR using rATG after kidney transplantation [10,11]. In the present study, we evaluated the effect of rATG on active AMR after kidney transplantation.

MATERIALS AND METHODS

Patients and Immunosuppressive Therapy

Between April 2013 and March 2018, 162 de novo consecutive adult kidney transplantations were performed with induction immunosuppressive therapy comprising extended-release tacrolimus (0.1 mg/kg once daily), mycophenolate mofetil (30 mg/kg/day twice daily), methylprednisolone (starting dose: 250 or 500 mg/day), and basiliximab (20 mg/day) at post-transplant days 0 and 4. Desensitization for ABO-incompatible and DSA-positive recipients was performed prior to transplantation with 0 to 4 times double-filtration plasmapheresis or plasma exchange and 1 to 2 times rituximab administration at a dose of 100 mg, according to the quantity of antibodies. All patients received a triple-drug combination of extended-release tacrolimus (trough level: 5.0 ng/mL), mycophenolate mofetil (1000 mg/day), and methylprednisolone (4 mg/day) for maintenance immunosuppressive therapy.

Diagnosis and Treatment of AMR

AMR was diagnosed based on episode biopsy findings according to the Banff criteria following the significant elevation of serum creatinine levels above baseline. A panel reactive antibody test was performed as a surrogate examination to detect the presence of DSA in all patients with active AMR. In case of panel reactive antibody positivity, a single-antigen beads test was performed to determine true DSA. The presence of DSA was used for the auxiliary diagnosis of AMR and was not essential for definite diagnosis according to the revised diagnostic criteria for AMR at the Banff 2017 Kidney Meeting Report [12].

For DSA-positive patients with active AMR, double-filtration plasmapheresis was performed twice to remove DSA prior to rATG administration. In case of acute active AMR diagnosed by clinical course and histopathological evidence according to biopsy results, steroid pulse therapy (methylprednisolone, 500 mg for 2 days) was administered prior to rATG administration.

For all patients with active AMR, 1.5 mg/kg/day of rATG was administered over 12 hours for 5 days following methylprednisolone (2 mg/kg/day), diphenhydramine (30 mg/day), and acetaminophen (400 mg/day). After the completion of a series of rATG administration, valganciclovir (450 mg/day 3 times/week for 3 months) was administered for prophylaxis against cytomegalovirus infection.

Study Design and Outcomes

We conducted a retrospective observational study of patients treated with rATG against active AMR. We analyzed patient characteristics, peripheral blood lymphocyte count, and adverse events as well as serum creatinine and urine protein levels as graft function indicators. This study was conducted in accordance with the principles of the Declarations of Helsinki and Istanbul and approved by the institutional review board.

Statistical Analysis

Statistical analysis was performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13], which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). Continuous variables were represented as means with standard deviations for parametric data or medians with ranges for nonparametric data. Serum creatinine and urine protein levels before and after rATG administration were assessed using paired t tests. A correlation analysis for nonparametric data was performed using Spearman's rank correlation coefficient. A statistically significant difference was considered when the 2-tailed P value was $< .05$.

RESULTS

Patient Characteristics

Of the 162 kidney transplant recipients, 13 (8.0%) developed active AMR within 302 (5–1743) days after transplantation. The characteristics of patients with active AMR are shown in Table 1. The mean age at transplantation was 50.3 ± 9.3 years. There were 3 (23.1%) patients with ABO-incompatible transplantation. Preformed DSA was detected prior to transplantation in 3 (23.1%) patients. Desensitization prior to transplantation was performed in 5 (38.5%) patients. Acute and chronic active AMR occurred in 9 (69.2%) and 4 (30.8%) patients, respectively. DSA was detected in 9 (69.2%) patients with active AMR.

Graft Function

The serum creatinine level significantly declined from 3.03 ± 1.19 mg/dL at peak prior to rATG administration to 1.68 ± 0.66 mg/dL at nadir after rATG administration (Fig 1). The median duration from rATG administration to reaching nadir serum creatinine level was 46 (10–329) days. The duration from rATG administration to reaching nadir serum creatinine level was positively correlated with the duration from transplantation to AMR ($r = .46$, $P = .11$) and duration from AMR to rATG administration ($r = .59$, $P = .035$). The urine protein level significantly declined from 3.01 ± 2.73 g/gCr at peak prior to rATG administration to 0.54 ± 0.52 g/gCr at nadir after rATG administration (Fig 2). The median duration from rATG administration to reaching nadir urine protein level was 106 (5–446) days. The duration from rATG administration to reaching nadir urine protein level was not correlated with the duration from transplantation to AMR ($r = .074$, $P = .81$); however, it was positively correlated with the duration from AMR to rATG administration ($r = .22$, $P = .46$).

Table 1. Characteristics of Patients With Active AMR

Patient	Age at Tx	Sex	Donor	ABO	Preformed DSA	Desensitization before Tx	Tx–AMR (d)	Acute/Chronic AMR	DSA at AMR
1	49	M	Living-unrelated	Com	N/D	None	14	Acute	+
2	47	F	Living-unrelated	Com	Detected	RXM + PP	12	Acute	+
3	46	F	Living-unrelated	Com	N/D	None	796	Acute	−
4	71	F	Living-unrelated	Com	N/D	None	100	Acute	−
5	62	F	Living-unrelated	Com	Detected	RXM + PP	5	Acute	+
6	46	M	Living-related	Incom	N/D	RXM + PP	302	Acute	+
7	55	F	Living-related	Incom	Detected	RXM + PP	7	Acute	+
8	49	M	Living-related	Com	N/D	None	358	Acute	−
9	36	F	Living-related	Com	N/D	None	63	Acute	−
10	46	M	Living-unrelated	Com	N/D	None	645	Chronic	+
11	59	M	Living-related	Incom	N/D	RXM + PP	1252	Chronic	+
12	47	M	Living-related	Com	N/D	None	450	Chronic	+
13	41	M	Living-related	Com	N/D	None	1743	Chronic	+

Abbreviations: AMR, antibody-mediated rejection; Com, compatible; DSA, donor-specific antibody; F, female; Incom, incompatible; M, male; N/D, not detected; PP, plasmapheresis; RXM, rituximab; Tx, transplantation.

Peripheral Blood Lymphocytes After rATG Administration

After rATG administration, peripheral blood lymphocytes (normal range: 900–4000/μL) instantly reduced from 1200 (100–2700)/μL to 0 (0–200)/μL (Fig 3). Thereafter, the peripheral blood lymphocyte count remained low at 750 (300–1500)/μL after 12 months and gradually recovered over a period of 12 months (Fig 3).

Adverse Events

No serious adverse event, such as death and graft loss, was observed. As an adverse event, fever occurred in 11 (84.6%) patients during the duration of rATG administration. The following hematological adverse events occurred: thrombocytopenia (n = 8, 61.5%), anemia (n = 4, 30.8%), and neutropenia (n = 2, 15.4%) within 3 months after rATG administration. Cytomegaloviremia occurred in 11 (84.6%) patients within 3 months after rATG administration; however, none of the patients who received valganciclovir as prophylaxis developed cytomegalovirus infection.

DISCUSSION

In the present study, we investigated the outcomes of rATG administration for the treatment of active AMR after kidney transplantation. Our results indicate that rATG administration is effective in kidney transplant recipients with active AMR.

rATG administration improved graft function by suppressing peripheral blood lymphocytes in kidney transplant recipients with acute and chronic active AMR. In 13 patients with acute or chronic active AMR, serum creatinine and urine protein levels declined after rATG administration. Consistent with our results, previous studies have reported that graft function recovered after rATG

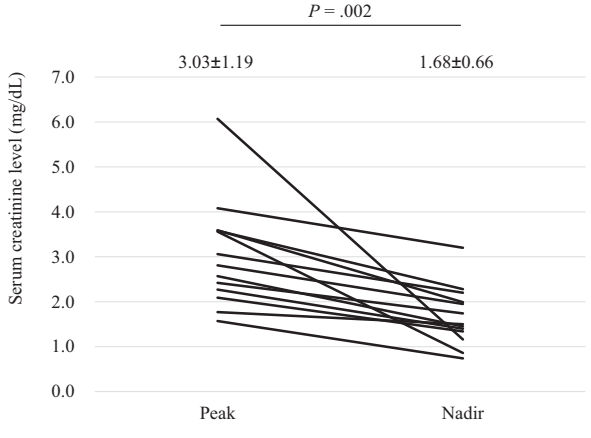

Fig 1. Change in serum creatinine level. Serum creatinine level significantly declined from peak before rabbit antithymocyte globulin administration to nadir after rabbit antithymocyte globulin administration.

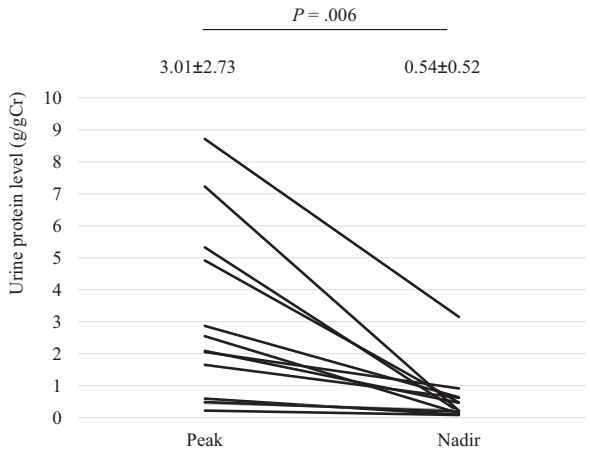

Fig 2. Change in urine protein level. Urine protein level significantly declined from peak before rabbit antithymocyte globulin administration to nadir after rabbit antithymocyte globulin administration.

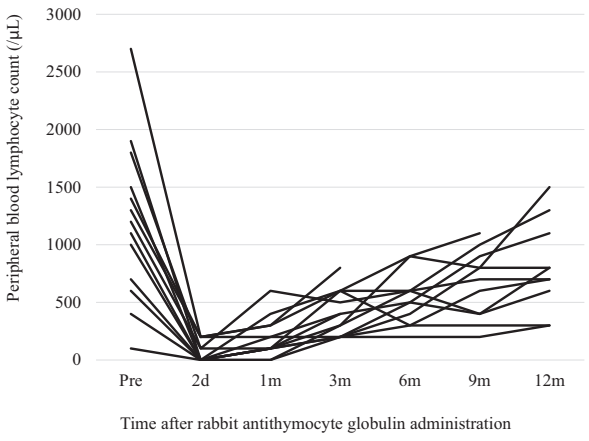

Fig 3. Time course of peripheral blood lymphocyte count after rabbit antithymocyte globulin administration.

administration in 7 patients with acute AMR [10] and 9 pediatric patients with chronic AMR [11]. In addition, our study indicated that rATG instantaneously reduced peripheral blood lymphocytes over a period of 12 months. The polyclonal antibody rATG can inhibit various types of lymphocytes containing B cells and plasma cells involved in antibody production. A previous in vitro study reported that rATG induces apoptosis of B cells and plasma cells via multiple pathways [14]. A previous clinical research indicated that a combination therapy including rATG affects memory B cells in the spleen [15]. Therefore, rATG may treat active AMR by inhibiting DSA production due to B cell lineage suppression.

There are several limitations of the study that should be acknowledged. This was a retrospective, single-center, and small-sized study. Therefore, the possibility of unintentional selection bias cannot be completely eliminated. In addition, observation was conducted only for a short period after rATG administration. Therefore, a long-term randomized controlled trial is necessary to clarify whether rATG can extend graft survival for patients with active AMR.

In conclusion, this study demonstrated that rATG is effective in kidney transplant recipients with acute and chronic active AMR. The rATG administration as a treatment for active AMR may contribute to positive graft outcomes after kidney transplantation.

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