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A comparative study of the ATP concentrations in the peripheral CD4$^+$ lymphocytes of living-donor and cadaveric-donor renal transplant recipients

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ABSTRACT

Renal transplant recipients receive immunosuppressive therapy to prevent acute rejection. However, immunosuppressive drugs are known to cause opportunistic infectious diseases. An ATP monitoring assay on the peripheral CD4$^+$ lymphocytes is useful for evaluating the risks of rejection and infection episodes in transplant recipients. The graft survival rate of cadaveric-donor renal transplant recipients has been reported to be lower than that of living-donor renal transplant recipients. Therefore, we compared the ATP concentrations in the peripheral lymphocytes of living-donor and cadaveric-donor renal transplant recipients. We measured the ATP concentrations in the peripheral lymphocytes of 17 living-donor and 7 cadaveric-donor renal transplant recipients every week for six weeks, and at three, six, and twelve months after transplantation. The ATP concentrations were measured using an ImmuKnow® assay kit. The rates of cytomegalovirus (CMV) infection and rejection episodes were also compared. With the exception of the concentrations at three weeks after transplantation, no significant difference was observed in the ATP concentrations of living-donor and cadaveric-donor renal transplant recipients. At three weeks, the ATP concentrations of living-donor recipients were significantly higher than those in the cadaveric-donor recipients ($p = 0.024$). However, the rates of both CMV infection and rejection episodes in living-donor and cadaveric-donor renal transplant recipients did not differ to a statistically significant extent during the twelve-month study period. Our data suggest that the ATP concentrations in the peripheral lymphocytes of living-donor and cadaveric-donor recipients were similar after renal transplantation (with the exception of the 3-week time-point). These observations suggest that the groups had similar risks of CMV infection and rejection until 12 months after transplantation.

KEYWORDS: ATP monitoring assay, cadaveric-donor, living-donor, renal transplantation

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INTRODUCTION

End-stage renal disease patients are mainly treated with dialysis therapy or renal transplantation. Renal transplantation is associated with a better quality of life and life expectancy. Renal transplant recipients receive immunosuppressive therapy to prevent acute rejection. However, immunosuppressive therapy is associated with the development of opportunistic infectious diseases. The cytomegalovirus (CMV) infection rate generally increases in renal transplant recipients due to over-immunosuppression.

The concentration of ATP in the peripheral $CD4^+$ cells is a useful biomarker for preventing infection and rejection episodes in transplant recipients undergoing immunosuppressive therapy. Indeed, ATP monitoring has been reported to have the potential to identify lung transplant recipients who are at risk of developing infectious diseases [1]. ATP monitoring has also been shown to be effective for predicting infection in kidney and heart transplant recipients [2, 3]. We have previously performed lymphocyte ATP monitoring in renal transplant recipients undergoing cyclosporine or tacrolimus-based immunosuppressive therapy [4]. The lymphocyte ATP concentrations in the peripheral blood of renal transplant recipients who were treated with cyclosporine-based immunosuppressive therapy were significantly lower than that of those who were treated with tacrolimus-based therapy. In parallel with these observations, the incidence of CMV infections in the patients who received cyclosporine-based therapy was higher than that in those who received tacrolimus-based therapy [4].

Renal transplant recipients may receive allografts from either living or cadaveric donors. However, the graft survival of cadaveric renal transplant recipients is lower than that of living-donor graft recipients [5]. Furthermore, the mortality rate of cadaveric-donor renal transplant recipients has been reported to be higher than that of living-donor recipients [5].

In the present study, the ATP concentrations in the peripheral $CD4^+$ lymphocytes of both living-donor and cadaveric-donor renal transplant recipients were monitored using the ImmuKnow® assay kit until 12 months after transplantation and compared. Furthermore, the rates of CMV infection and rejection episodes in living-donor and cadaveric-donor renal transplant recipients were compared until 12 months after transplantation.

MATERIALS AND METHODS

Patients

The present study was approved by the Ethics Review Board of the Medical Faculty of Niigata University, and all patients included in this study provided their written informed consent. Heparinized venous blood was obtained from 24 renal transplant recipients (living donors, n = 17; cadaveric donors, n = 7) before transplantation (not performed in cadaveric-donor recipients) and every week for six weeks and at three, six, and twelve months after transplantation. The characteristics of renal transplant recipients are shown in table 1. The mean age of the living-donor renal transplant recipients was 35.2 ± 13.6 years, while that of the cadaveric-donor renal transplant recipients was 48.9 ± 11.3 years. There was a significant difference in the ages

Table 1. The characteristics of the living-donor and cadaveric-donor renal transplant recipients.

Donor type	Living donor (n = 17)	Cadaveric donor (n = 7)	P value
Mean age (years) ± SD	35.2 ± 13.6	48.9 ± 11.3	p = 0.026
Male/Female	12/5	5/2	NS
Cyclosporine/Tacrolimus	8/9	5/2	NS
HLA-AB mismatch number ± SD	2.1 ± 1.0	2.6 ± 1.3	NS
HLA-DR mismatch number ± SD	1.2 ± 0.5	0.7 ± 0.5	NS

Age, HLA-AB and HLA-DR were assessed using the Mann-Whitney *U* test.
Sex ratios, either based on cyclosporine or tacrolimus were assessed using Fisher's exact probability test.

of the living-donor and cadaveric-donor recipients. The living-donor recipients included 12 male patients and 5 female patients. The cadaveric-donor recipients included 5 male patients and 2 female patients. Eight of the 17 living-donor renal transplant recipients received primary cyclosporine-based immunosuppressive therapy; the other 9 patients received tacrolimus-based therapy. Five out of the 7 cadaveric-donor renal transplant recipients received primary cyclosporine-based immunosuppressive therapy; the other two received tacrolimus-based therapy. The mean human leukocyte antigen (HLA)-AB mismatch numbers in the living-donor and cadaveric-donor recipients were 2.1 ± 1.0 and 2.6 ± 1.3, respectively; the mean HLA-DR mismatch numbers were 1.2 ± 0.5 and 0.7 ± 0.5. All of the transplant recipients received renal allografts from living donors and cadaveric donors after blood sampling for analysing the ATP concentrations in the peripheral $CD4^+$ cells. All of the recipients underwent renal transplantation during the period November 2010 to December 2011 at Niigata University Medical and Dental Hospital.

Immunosuppressive therapy

After renal transplantation, the patients were primarily treated with maintenance immunosuppressive therapy, which included a combination of either cyclosporine (Neoral cap., Novartis Pharma Co., Switzerland) or tacrolimus (Prograf cap., Astellas Co., Japan) with basiliximab (20 mg; Simulect, Novartis Pharma Co., Switzerland) on days 0 and 4, plus methylprednisolone and mycophenolate mofetil (Celcept [250 mg, Cap.] Chugai Co., Japan). Two patients who received tacrolimus-based immunosuppressive therapy did not receive basiliximab. The starting doses of cyclosporine were 2-3 mg/kg/day (intravenous) or 8 mg/kg/day (oral). The starting dose of tacrolimus was 0.05 mg/kg/day (intravenous) or 0.2 mg/kg/day (oral). The starting dose of methylprednisolone was 125 mg/day, while that of mycophenolate mofetil was 1000 or 2000 mg (b.i.d).

Monitoring of ATP concentrations using the ImmuKnow® assay kit

The immune cell function of the patients was estimated based on the ATP concentrations in their $CD4^+$ cells, which were measured using the ImmuKnow® assay (Cylex Inc., Columbia, USA), an FDA-approved test. Peripheral blood samples were collected into a sodium heparin-containing tube, and the intracellular concentrations of ATP were measured. All of the blood samples were processed for the measurement of ATP on the day of sample collection. Briefly, 250 μL of anticoagulated whole blood was diluted with the enclosed sample diluent in the ImmuKnow® assay kit to a final volume of 1000 μL. Samples were added to the wells of a 96-well plate and incubated with phytohemagglutinin for 15-18 h (37 °C, 5% CO_2). After the enrichment of the $CD4^+$ T cells by the addition of magnetic particles coated with an anti-human CD4 monoclonal antibody (Dynabeads; Dynal, Oslo, Norway), the blood cells were washed and lysed to release the intracellular ATP. The released ATP was measured by a luciferin/luciferase assay using a luminometer. The patient's immune response was expressed as the concentration of ATP (ng/ml) [1-4, 6-10].

In the present study, we measured the ATP concentrations in the peripheral $CD4^+$ cells in the living-donor and cadaveric-donor renal transplant recipients every week for six weeks and at three, six, and twelve months after transplantation. The ATP concentrations immediately before the transplantation could only be measured in the living-donor recipients. The ATP concentrations in living-donor and cadaveric-donor renal transplant recipients were compared.

Reagents

The ATP concentrations were determined using an ImmuKnow® kit (Cylex Inc., Columbia, USA).

Statistical analysis

The variations in the ATP concentrations in the peripheral $CD4^+$ cells of living-donor and cadaveric-donor recipients were examined using the Mann-Whitney *U* test. The variations in the ATP concentrations of each group were analyzed with Bonferroni multiple comparison tests at the above-mentioned time points. The differences between the two groups in the rates of CMV infection and rejection episodes were assessed using Fisher's exact probability test. These data analyses were

performed using the PASW statistics base 18.0 (SPSS Japan Inc. an IBM company), GraphPad Prism 6 GraphPad Software Inc., USA) and Excel 2016 (Microsoft) software programs.

RESULTS

The ATP concentrations in the peripheral $CD4^+$ lymphocytes were determined using an ImmuKnow® kit before transplantation (in the living-donor recipients only), then each week for six weeks, and at three, six, and twelve months after transplantation. Figure 1 shows the mean ATP concentrations in the 17 living-donor recipients in comparison to the 7 cadaveric-donor recipients after renal transplantation. In both the living-donor and the cadaveric-donor renal transplant recipients, the ATP concentrations increased until 2 and 3 weeks after transplantation. However, the ATP concentrations gradually decreased until 12 months after transplantation. Generally, there were no significant differences in the ATP concentrations of the living-donor and the cadaveric-donor recipients. However, at three weeks after transplantation, the median ATP concentration of the living-donor recipients was significantly higher than that of the cadaver-donor recipients ($p = 0.024$).

The ATP concentrations in the living-donor recipients before transplantation were compared with those after transplantation. The median ATP concentrations at two and three weeks after transplantation were significantly higher than the median ATP concentration before transplantation ($p = 0.008$, $p = 0.01$), whereas the median ATP concentration at 12 months after transplantation was not significantly lower than that before transplantation. However, in cadaveric-donor renal transplantation recipients, the ATP concentrations at 12 months after transplantation were not significantly different from those measured at the other points of time. The incidence of clinical events after transplantation

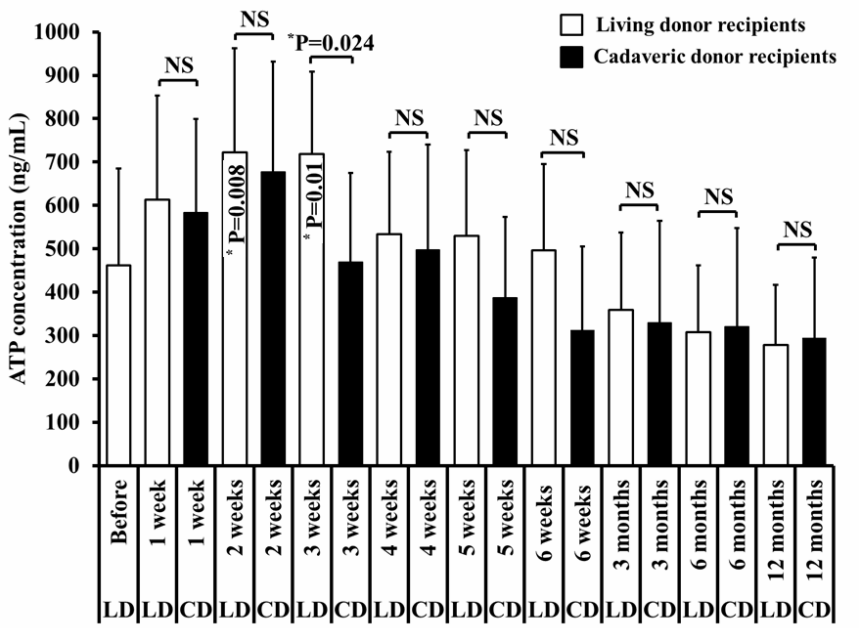

Figure 1. A comparative study of the ATP concentrations in the peripheral $CD4^+$ cells of living-donor and cadaveric-donor renal transplant recipients before transplantation (living-donor recipients only), and at each week for six weeks, and at three, six, and twelve months after transplantation. A statistically significant difference was observed in the ATP concentrations of the living-donor and cadaveric-donor renal transplant recipients at 3 weeks after transplantation (*p = 0.024). A one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test was used for the statistical analysis of the ATP concentrations before and after renal transplantation. In the living-donor recipients, only the ATP concentrations at 2 weeks (*p = 0.008) and 3 weeks (*p = 0.01) after transplantation were significantly higher than the pre-transplantation values. In the case of cadaveric renal transplantation, the ATP concentrations did not differ to a statistically significant extent at any time-point after transplantation.

Table 2. The clinical events of the cadaveric and living-donor renal transplant recipients.

Donor types	CMV infection rate (%)	CMV infection episode (day after transplantation)	
Living donor (n = 17)	8/17 (47.1%)	27, 34, 34, 34, 41, 41, 52, 62	NS
Cadaveric donor (n = 7)	4/7 (57.1%)	18, 22, 32, 92	
Donor types	**Rejection rate (%)**	**Rejection episode (day after transplantation)**	
Living donor (n = 17)	3/17 (17.6%)	7, 34, 38	NS
Cadaveric donor (n = 7)	1/7 (14.3%)	63	

The CMV infection and rejection rates were assessed by Fisher's exact probability test.

in the living-donor and cadaveric-donor recipients is shown in table 2. CMV infections occurred in 8 of the 17 living-donor recipients (47.1%) on days 27, 34, 34, 34, 41, 41, 52 and 62, and in 4 of the 7 cadaveric-donor recipients (57.1%) on days 18, 22, 32 and 92. Acute allograft rejection occurred in 3 of the 17 living-donor recipients (17.6%) on days 7, 34 and 38, and in 1 of the 7 cadaveric-donor recipients (14.3%) on day 63. The rates of CMV infection and rejection episodes in the living-donor and the cadaveric-donor recipients did not differ to a statistically significant extent (Table 2). There were no cases of graft loss or death due to transplantation at the end of the 12-month study period.

DISSCUSSION

In the present study, we compared the ATP concentrations in the $CD4^+$ lymphocytes of the peripheral blood in consecutive living-donor and cadaveric-donor renal transplant recipients each week for six weeks, and at three, six, and twelve months after transplantation. The ImmuKnow® kit, which was used to measure the ATP concentrations, uses phytohemagglutinin (PHA)-L as a mitogen. PHA-L only activates $CD4^+$ T cells. Thus, the ATP assay uses activated peripheral blood mononuclear cells (PBMCs) to monitor a patient's immunological status [1-4, 6-10].

The measurement of the ATP concentrations in peripheral $CD4^+$ cells has been reported to be useful for monitoring the risk of infection and acute rejection episodes after renal transplantation [2]. Low ATP concentrations (< 225 ng/mL) have previously been suggested to be associated with an increased risk of CMV infection, while moderate to high ATP values (> 225 ng/mL) were associated with a reduced risk of CMV infection in renal transplant recipients [2]. On the other hand, high ATP concentrations (> 525 ng/mL) were suggested to be associated with an increased risk of acute rejection [2]. Husain *et al.* reported that ATP monitoring has the potential to identify lung transplant recipients who are at risk of developing infectious diseases [1]. In contrast, López *et al.* reported that although the use of the ImmuKnow® assay for assessing the risk of infection had been described in a report about post-transplant CMV infection-related lymphoproliferative disorder, high ATP values, as estimated by the ImmuKnow® kit, did not indicate an increased risk of acute rejection [6].

We have previously performed lymphocyte ATP monitoring in renal transplant recipients undergoing cyclosporine- or tacrolimus-based immunosuppressive therapy [4]. The lymphocyte ATP concentrations in the peripheral blood of renal transplant recipients who were treated with cyclosporine-based immunosuppressive therapy were significantly lower than those of the recipients who were treated with tacrolimus-based therapy. The incidence of CMV infection in the recipients who received cyclosporine-

based therapy was higher than that in those who received tacrolimus-based therapy [4]. Thus, the ATP concentrations, as determined by the ImmuKnow® kit, can be a useful biomarker for monitoring the risk of CMV infection in renal transplant recipients.

Infection control is a very important part of individual therapeutic care to ensure graft survival. Koukoulaki *et al.* reported that the donor age was correlated with allograft survival and that it was a potential prognostic factor [11]. Furthermore, they showed that panel reactive antibodies influenced long-term allograft survival, while HLA mismatches were not correlated with graft survival [11]. The main problem for long-term graft survival in renal transplant recipients is recent CMV infection as opposed to acute rejection due to over-immunosuppression. Allograft survival is not only influenced by appropriate immunosuppressive treatment but also by other factors, including donor age, panel reactive antibodies, and the donor type (living or cadaveric).

The risk of graft loss in cadaveric-donor renal transplant recipients is generally higher than that in living-donor renal transplant recipients. In the present study, the rate of CMV infection and rejection episodes in living-donor and cadaveric-donor recipients did not differ to a statistically significant extent during the 12-month study period. Furthermore, the ATP concentrations in the $CD4^+$ cells of the living-donor and cadaveric-donor recipients were similar throughout the 12-month study period.

The ATP concentrations increased until 2 weeks after transplantation in both living-donor and cadaveric-donor renal transplant recipients. However, these ATP concentrations tended to decrease from week 3 until 12 months after both types of transplantation. Furthermore, at 12 months after transplantation, the ATP concentrations of the living-donor recipients were lower than the ATP concentrations before transplantation. Thus, the greatest rejection risk was suggested to occur at 2 weeks after transplantation. On the other hand, the risk of opportunistic infectious diseases increased from week 3 until 12 months after transplantation in both the living-donor and the cadaveric-donor renal transplant recipients.

There was a significant difference in the ages of the living-donor and the cadaveric-donor renal transplant recipients (Table 1). However, we have previously reported that the ages of dialysis and chronic kidney disease (CKD) patients did not correlate with the ATP concentrations [10]. Furthermore, the stimulation indices of peripheral blood mononuclear cells, as reflected by concanavalin A, and the ages of the renal transplant recipients were not correlated, and the ability of immune cells to proliferate in response to T cell mitogens appeared not to be influenced by age [12]. There were no cases of graft loss or patient death during the 12-month study period. These observations suggest that the living-donor and cadaveric-donor renal transplant recipients showed similar risks of CMV infection and rejection until 12 months after transplantation.

CONCLUSION

The ATP monitoring assay was capable of evaluating the risks of infection and rejection in living-donor and cadaveric-donor renal transplant recipients. With the exception of the measurements that were taken at three weeks after transplantation, the ATP concentrations of the living-donor and cadaveric-donor renal transplant recipients were similar until 12 months after transplantation. Graft survival in living-donor recipients is generally longer than that in cadaveric-donor transplant recipients. However, the risks of infection and rejection, as assessed using an ATP monitoring assay, were similar in living-donor and cadaveric-donor renal transplant recipients. Thus, our present data suggest that the risks of CMV infection and rejection in living-donor and cadaveric-donor renal transplant recipients were similar until 12 months after transplantation.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest in association with the present study.

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