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Increased intracellular adenosine triphosphate level as an index to predict acute rejection in kidney transplant recipients

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ABSTRACT

Background: Peripheral blood CD4+ T cell adenosine triphosphate (ATP) release has been reported to be an adjunct tool to evaluate global cellular immune response in solid-organ transplant recipients. However, the correlation between the ATP level and rejection was controversial. The aim of this prospective clinical study was to explore the association between the intracellular ATP level and the occurrence, progression, and treatment of acute rejection (AR) episodes, determine the predicting value of intracellular ATP level for AR in kidney transplant (KT) recipients. Patients and methods: In the period of October 2011 to October 2012, 140 KT recipients were recruited and followed for six months after transplantation. Patients were categorized into stable group and AR group according to their clinical course. Whole blood samples were collected pretransplantation, and at 7, 14, 21, and 28 days, and at 2, 3, 4, 5 and 6 months post-transplantation. Additional blood samples were obtained from AR patients on the day AR occurred, on the day before and 3 and 7 days after intravenous anti-rejection therapy started, and on the day when AR reversed. The intracellular ATP in CD4+ T cells was detected by ImmuKnow Immune Cell Function Assay according to the manufacturer's instruction. The absolute number of CD4+ T cells and the trough levels of tacrolimus and cyclosporine were also measured. Results: The ATP level detected on the day AR occurred (627.07 ± 149.85 ng/ml) was obviously higher than that of the stable group (320.48 ± 149.11 ng/ml, $P < 0.05$). ATP value decreased to 265.35 ± 84.33 ng/m at the end of anti-rejection therapy, which was obviously lower than that measured on the day before the anti-rejection therapy started (665.87 ± 162.85 ng/ml, $P < 0.05$). ROC analysis revealed that increased intracellular adenosine triphosphate level showed better sensitivity and specificity than those obtained using single time point detection (89.5% vs 85.0%;95.0% vs 88.9%). The best cutoff value was 172.55 ng/ml. A positive correlation between the intracellular ATP level and absolute CD4+ T cell number ($r = 0.656$, $P < 0.001$) was found in the patients with CD4+ T cell counts <200/μl.

1. Introduction

Kidney transplantation has been successfully used as an effective therapeutic option for patients with end-stage kidney diseases. With the introduction of a variety of immunosuppressive agents, the graft and patient survival rate after kidney transplantation has been significantly improved during the past decade. However, acute rejection (AR), which usually occurs on days 7–14 in the early post-transplantation period [1,2], remains a major obstacle for the long term survival in kidney transplant (KT) recipients. Currently, allograft rejection is taken into consideration only when continuous and irreversible deterioration of renal function occurs, and the gold standard-biopsy procedure usually takes up several days. As a result, anti-rejection therapy starts relatively late as kidney damage has already been established. Hence, convenient and noninvasive approaches for predicting and diagnosing AR would be of considerable value to improve outcomes in KT recipients.

The Cylex ImmuKnow Cell Function Assay may be a promising tool for post-transplant immune monitoring. In 2002, the U.S. Food and Drug Administration (FDA) approved this assay for evaluating global cellular immune response in solid-organ transplant recipients [3]. It assesses the activity of $CD4^+$ T cells by measuring the concentration of intracellular adenosine triphosphate (ATP) after stimulation with phytohemagglutinin (PHA) in vitro. It is expected that this assay could be beneficial in monitoring the net immune state in transplant recipients and lead to better patient management and evidence-based individualization.

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Based on a multicenter study in American, Kowalski et al. classified immune response of transplant recipients into three zones: strong (≥525 ng/ml ATP), moderate (226–524 ng/ml ATP), and low (≤225 ng/ml ATP) [4]. They reported that ATP values higher than 525 ng/ml were associated with an increased risk of AR. Thereafter, most of the following studies used ATP level at 525 ng/ml as a threshold to investigate the predictive role of intracellular ATP values at single time point for AR. Several studies have shown that the high intracellular ATP levels can identify patients at risk for rejection [5–7], whereas others argue against its predictive capacity [8]. Therefore, the correlation between ATP level and rejection is still undetermined.

2. Objective

We hypothesized that patients with high intracellular ATP level in stimulated $CD4^+$ T cells measured by the Cylex ImmuKnow Cell Function Assay are at increased risk of AR episodes. The aim of this prospective clinical study was to explore the association between the intracellular ATP level and the occurrence, progression, and treatment of AR episodes.

3. Materials and method

3.1. Patients

This is a single center prospective study that was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Medical College of Xi'an Jiaotong University. The informed consent was obtained from all study participants. A total of 140 KT recipients were recruited from October 2011 to October 2012, and all were first-time transplant recipients. All kidney grafts were from either living donors or cardiac deceased donors. Patients were excluded when suffered infection or delayed graft function during the six month follow-up after transplantation.

All patients received induction therapy with rabbit anti-human T lymphocyte immunoglobulin (ATG) and steroids. Subsequent immunosuppressive maintenance regimens were usually standard triple therapy, which consisted of tacrolimus (FK506) or cyclosporine A (CsA), combined with mycophenolate (MMF) and prednisone.

AR was defined clinically by an acute rise in serum creatinine and urine output decrease, which was confirmed by a kidney allograft biopsy. Rejection episodes were treated with methylprednisolone at a dose of 500 mg by intravenous injection once daily for three consecutive days. ATG was used in the patients with refractory acute rejection episodes.

3.2. Sample collection

The anticoagulated whole peripheral blood samples were collected from all recipients pre-transplantation, and at 7, 14, 21, and 28 days, and at 2, 3, 4, 5 and 6 months post-transplantation. Additional blood samples were obtained from AR patients on the day AR occurred, on the day before and 3 and 7 days after intravenous anti-rejection therapy started, and on the day when AR reversed.

3.3. Cylex immune cell function assay analysis

The intracellular ATP in $CD4^+$ T cells was detected by Cylex ImmuKnow Cell Function Assay (Cylex, Inc., Columbia, MD, USA) according to the manufacturer's instruction. Briefly, 100 μl whole blood (1:4 dilution) was added to wells of a 96-well microtiter plate, and incubated overnight (15–18 h) with or without PHA at 37 °C in a 5% CO_2 incubator. On the following day, $CD4^+$ T cells were selected by anti-human CD4 monoclonal antibody-coated magnetic particles (Dynal, Oslo, Norway) and a strong magnet (Cylex Magnet tray 1050; Cylex Inc., Columbia, MD, USA), washed and lysed to release intracellular ATP. Subsequently, a luciferin/luciferase reagent was added and released ATP was measured by a luminometer (Turner Biosystems, Sunnyvale, CA, USA). The concentration of ATP was expressed as ng/ml.

3.4. Flow cytometry and absolute $CD4^+$ T cell counting

Before intracellular ATP detection, part of the blood samples were stained for lymphocyte surface markers and then sorted by flow cytometry (FACSCalibur, BD, USA) to obtain the ratio of $CD4^+$ T lymphocytes to the total peripheral blood leukocytes, which was then used to calculate the absolute number of $CD4^+$ T cells by multiplying the total leukocyte number. Blood routine tests were performed to obtain the leukocyte number in the blood samples. FITC-labeled mouse anti-human $CD3^+$, PE-labeled mouse anti-human $CD4^+$ and PE-labeled mouse anti-human $CD8^+$ monoclonal antibodies were from BD (USA).

3.5. Immunosuppressive agent concentration monitoring

The trough levels of tacrolimus and cyclosporine were measured in the whole blood samples using a microparticle enzyme immunoassay (MEIA) according to the manufacturer's instructions (Abbott Diagnostics, North Chicago, IL, USA).

3.6. Statistical analysis

All data were recorded as the mean ± standard deviation. Continuous variables were compared using Student's *t* test. Categorical variables were compared using the chi-square or the Fisher's exact test. Correlations between the intracellular ATP and other variables were determined using the Pearson's correlation coefficients, with correlation expressed as r values. Receiver operator characteristic (ROC) analysis was performed to assess the potential of intracellular ATP for distinguishing patients with and without rejection. All statistical calculations and tests were performed using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA), GraphPad Prism software (GraphPad Prism Software Inc., San Diego, CA, USA), and MedCalc (MedCalc Software, Mariakerke, Belgium). A *P* value less than 0.05 was considered statistically significant.

4. Results

4.1. Intracellular ATP level in Chinese healthy adults and kidney transplant recipients

At the endpoint of this follow-up, 19 patients experienced AR episodes and 121 patients were categorized into stable group. Demographic details for these patients are

Table 1
Baseline characteristics of the kidney transplant recipients.

Variable	SG (n = 121)	ARG (n = 19)	*P* value
Recipient age(year; mean ± SD)	38.70 ± 7.20	31.90 ± 5.23	0.46
Gender (male; female)	89:32	12:7	0.41
Donor age (year; mean ± SD)	52.1 ± 8.75	45.1 ± 6.27	0.76
Type of transplant			0.31
Deceased donor	72	12	
Living related	49	7	
Pre-transplant PRA, n (%)			0.09
PRA < 10%	120	17	
10% ≤ PRA < 50%	1	2	
HLA A, B, DR MM (number)	2.45 ± 0.75	2.53 ± 0.46	0.37
Cold ischemia time (hour)	0.98 ± 0.43	1.03 ± 0.57	0.95
Immunosuppression protocol			0.78
CsA + MMF + Pred	32	4	
FK + MMF + Pred	89	15	

Abbreviations: SG, stable group; ARG, acute rejection group; PRA, panel reaction antibody; MM, mismatches; HLA, human leukocyte antigen; FK506, tacrolimus; MMF, mycophenolate mofetil; Pred, prednisone; CsA, cyclosporine A.

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