Intracellular ATP Production By CD4 T Cells: Comparison of Different Conditions of Cells Sources And Clinical Utility In Renal Transplantation

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Introduction

The immunosuppression is essential to have success in transplantation. The immunosuppression is mandatory to prevent allograft rejection. The aim is to avoid over-immunosuppression (because the patient could develop an infection or tumour) or under-immunosuppression (because it could cause a rejection of the graft). The recipient will need immunosuppressive drugs for life, so immunosuppression monitoring is essential to get the optimal levels of drug. Pharmacokinetic and pharmacodynamics data allow to personalize the treatment to each patient. Pharmacokinetics analyse what the organism does with the drug, which is the absorption, distribution, metabolization and excretion of the drug, whereas pharmacodynamics study how the drug affects the organism. The ImmuKnow®-the Cylex® Immune Cell Function Assay is an easy and rapid kit to detect cell-mediated immunity by measuring the concentration of adenosine triphosphate (ATP) from CD4+ cells following stimulation. This method requires complete fresh blood and permit obtain results in just 24 hours. It's based on a stimulation with a polyclonal stimulus for T Lymphocytes, phytohemagglutinin (PHA), and value the intracellular ATP concentration of CD4+ cells during an early stimulation. The method isolate the cells with beads covered with anti-CD4+. The ATP reacts with luciferin/luciferase after cellular lysis and the concentration is measured by a luminometer. Low concentration of intracellular ATP on CD4+ indicates high load of immunosuppression and increased risk of infection, and high values indicate risk of rejection. Intracellular ATP could be used as a biomarker because it indicates the level of activation of CD4+ lymphocytes, which are involved in the immune response of rejection and infection.

Aims

To compare the efficiency of the ImmuKnow®-the Cylex® Immune Cell Function Assay with complete blood and peripheral blood mononuclear cells (PBMC) in different conditions of storage. To evaluate the performance of ImmuKnow® in a subset of renal transplant patients suffering of biopsy-proven acute rejection.

Methods

- **Samples:** This work has been performed with blood collected in heparin sodium from adult healthy donors. Each sample was tested in six different conditions: Fresh blood, fresh blood after been stored 24 hours at room temperature, fresh blood after been stored 24 hours at 4°C, fresh PBMC, PBMC after been stored 24 hours at 4°C and PBMC after one week frozen. Additionally, we studied 6 renal transplant patients with biopsy-proven acute rejection. Blood samples to perform ImmuKnow® in fresh complete blood were collected at admission for biopsy because of clinical suspicion of acute rejection and at 10 and 30 days after rejection.
- **PBMC Extraction:** The PBMC were isolated by Ficoll-Hypaque density centrifugation and collected in R10 medium.
- **Freezing of PBMC:** The PBMC were frozen using FBS-RPMI 1:1 with 10% dimethil sulphoxide using CoolCell® Alcohol-Free Cell Freezing Containers a -80°C (Alcohol-free controlled-rate -1°C/minute cell freezing containers).
- **ImmuKnow®-the Cylex® Immune Cell Function Assay:** The whole blood samples were tested following the instructions of the manufacturer. The test was adapted to PBMC at final concentration of $2.5x10^5$ cells/well instead of the diluted whole blood. The CD4+ cells were isolated with magnetic beads after stimulation with PHA. Intracellular ATP was released by cellular lysis and measured by luciferin/luciferase reaction in a luminometer (Figure 1).
- **Statistical analysis:** Data have been analyzed using GraphPad Prisms 5.03 software (San Diego, CA) and medians were compared using Mann-Whitney and Wilcoxon test.

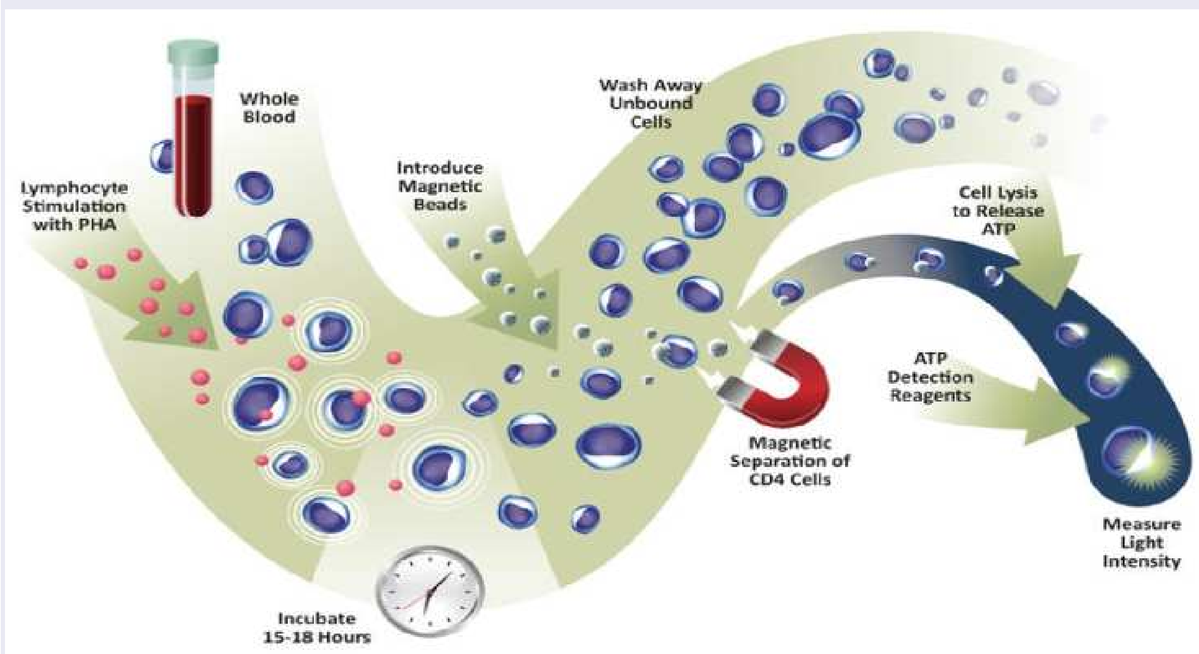

Figure 1 : Summary of the Immuknow test.

Results

Whole blood from 6 healthy controls were processed in three different conditions of storage in order to assess the reproducibility of the test (ImmuKnow®-the Cylex®). No differences in ATP production in fresh blood and room temperature-stored blood was observed (Figure 2A) nevertheless a decrease in ATP production after 4°C storage was demonstrated (Figure 2A). In the laboratory another source of lymphocytes is performed after isolation upon Ficoll to get PBMC, the next step was to compare the results of ATP production of fresh blood and PBMC. The median of ATP production in fresh blood was 250 ng/mL whereas in PBMC only achieved 50 ng/mL (Figure 2B).

After these results we decided to use whole blood for the study. Five samples from renal transplant patients were monitored before biopsy, 10 and 30 days post biopsy. A decrease in the median of ATP production was observed from the moment of the biopsy and 30 days post-biopsy (355 vs 303 vs 284 at biopsy, 10 and 30 days post-biopsy respectively) (Figure 3).

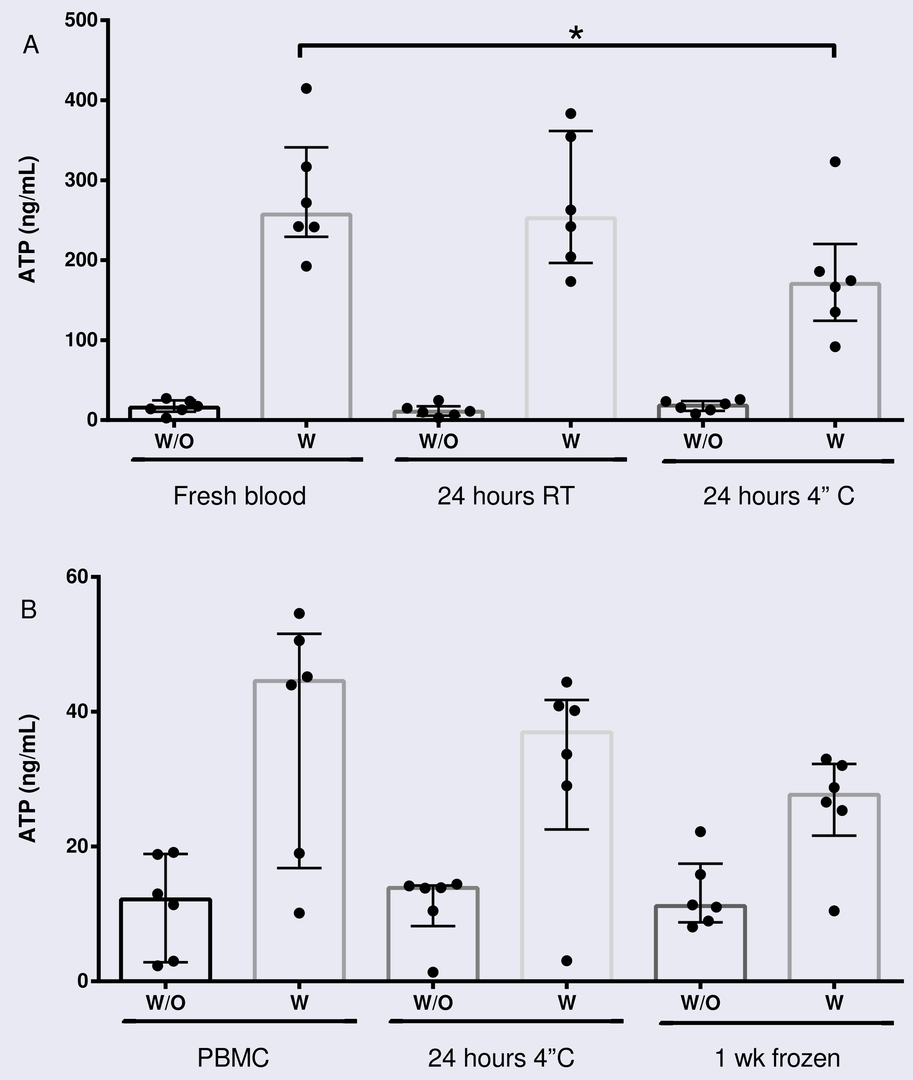

Figure 2 : Intracellular ATP concentration of lymphocytes analyzed in different conditons. Whole blood (A) and PBMC (B) processed under three different conditions were tested, fresh whole blood or PBMC (bars on left hand-side), stored at room temperature (RT) bars on the center and at 4°C for whole blood and 1 week (wk) frozen for PBMC (bars on right hand-side). The medians in columns and interquartilic range are depicted, medians were compared using U-Mann Whitney test ($p<0.05$ was considered significant).

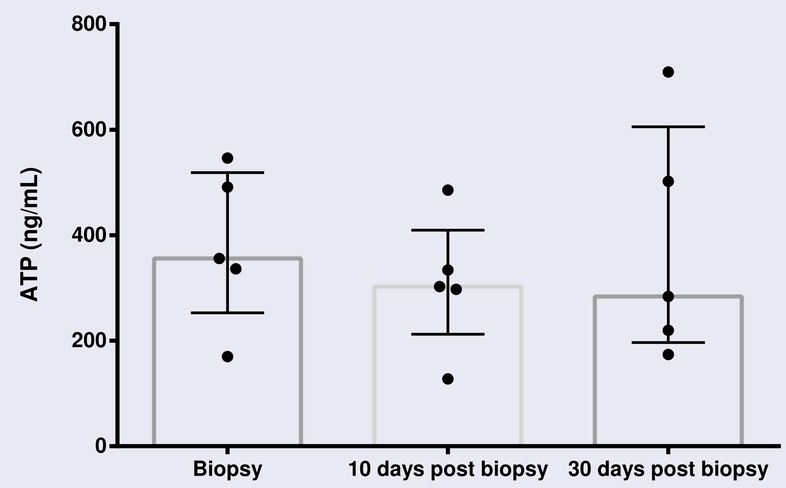

Figure 3 : Intracellular ATP concentration in renal transplant patients. Levels of ATP production were measured at the time of biopsy (left), 10 days (center) and 30 days post biopsy (right). The medians were compared using Wilcoxon test. No differences in medians were observed at any timepoint.

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Conclusions

ImmuKnow is only reliable with fresh blood and comparable after storage 24 hours at room temperature. The decreased level of ATP production in renal transplantation after diagnosis of rejection could be explained due to an increase of immunosuppressive load for acute rejection treatment.