

Abstract

Complications of Calcineurin inhibitor (CNI), in particular nephrotoxicity, have a major effect on morbidity and mortality within the transplant setting. We randomized liver transplant patients with renal dysfunction under CNI treatment to either a) receive mycophenolate mofetil (MMF) up to a dose of 2 g/day followed by consecutive reduction of CNI (CNI reduction group) or b) to continue their current CNI dose (control group). Peripheral blood mononuclear cell (PBMC) were isolated from patient blood at baseline and after conversion to MMF/low dose CNI regimen. The expression of CD3, CD4, CD8 on PBMC and the expression of activation marker was determined by FACS analysis. In *in vitro* experiments freshly isolated PBMC from healthy volunteers were treated with mycophenolic acid (MPA) (1 up to 10 μ M), cyclosporine A (CsA) (25-100 ng/mL), tacrolimus (Tac) (2-10 ng/mL) and combined CsA/MPA or Tac/MPA. T cell proliferation was measured by carboxyfluorescein diacetate succinimidyl ester and FACS analysis. T cell cycle analysis was performed by bromodexoxyuridine staining and FACS. Furthermore activation marker and nuclear factor of activated T cell (NFAT)-regulated cytokine expression was analyzed by FACS. The expression of cytokine mRNA was measured by real-time RT PCR. Combined MMF and low dose CNI therapy leads to reduction of percentages of CD8⁺ T cells, CD4⁺CD45RO⁺ T cells, CD3⁺CD56⁺ T cells and inhibition of activation marker expression. *In vitro* experiments have demonstrated that 1 μ M MPA completely blocks the proliferation of CD4 and CD8 T cells. MPA stops the cell cycle of activated T cells at G0/G1 phase and this effect is dose-dependent. Both MPA and CNI inhibit the expression of activation T cell markers and this effect is potentiated by combined exposure to MPA and CNI. MPA has no detectable effect on NFAT-regulated genes such as IL-2, IL-4, IFN- γ and their protein expression. Our results suggest that FACS analysis and assessment of cytokine expression of PBMC represent important tools for monitoring and optimizing the immunosuppressive regimen within the liver transplant setting. Adjustment of the MMF dosage by detection of the AUC may be required to avoid over-immunosuppression. Combined MMF and low dose CNI exhibit anti-proliferative effects in T cells by a) blocking the S phase of the cell cycle, b) decreasing NFAT-regulated gene and activation marker expression.

Key Words: Calcineurin inhibitor, transplantation, MMF, T lymphocyte