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Tacrolimus in combination with FTY720 — an analysis of renal and blood parameters☆

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Abstract

Calcineurin inhibitors (CNIs) are routinely used in immunosuppressive therapy and both Cyclosporine (CsA) and Tacrolimus (FK506) show similar efficacies to prevent rejection and death within the first year after organ transplantation. However, their use is limited by side effects such as kidney damage, hypertension, onset of diabetes and hyperlipidemia. It is a consensus that compared with CsA, FK506 causes less changes in blood pressures, serum lipids and renal function. Nevertheless, FK506 use is associated with a higher incidence of post-transplant diabetes mellitus (PTDM). FTY720 is a new compound that has shown a protective effect in animal models with respect to rejection in transplantation, ischemia–reperfusion injury, autoimmune diseases and tumor development. FTY720 acts by altering lymphocytes homing from blood to peripheral lymphoid organs. In mice, FTY720 administered in combination with CsA during 21 days has prolonged skin allograft survival without causing significant renal changes. In a model of CsA-induced chronic nephropathy in rats, FTY720 administration prevented renal injury suggesting benefit from using a combination of these drugs. In a canine kidney allograft model, FTY720 in combination with low doses of CsA or FK506 showed an addictive anti-rejection effect without causing critical adverse effects. We therefore, investigated whether 21 days of FTY720 administration in association with FK506 could prevent renal damage and development of diabetes in mice. Mice receiving FK506 alone or FTY720+FK506 during 21 days showed changes in kidney function and structure besides an increase in blood glucose and lymphopenia. The FTY720+FK506 combination requires further investigation with an aim toward understanding the mechanisms involved with respect to side effects.

Keywords: Transplantation; Immunosuppression; Renal function; Nephrotoxicity

1. Introduction

Immunosuppressive drugs have been used with success to improve allograft survival and reduce the risk of acute rejection. However, chronic rejection, infection and drug toxicity remain as the major causes of morbidity and mortality in the transplanted population.

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Cyclosporine (CsA) and Tacrolimus (FK506) are calcineurin inhibitors (CNIs) with immunosuppressive properties and broad clinical applicability. However, their use is limited by side effects such as kidney damage, hypertension, onset of diabetes, and hyperlipidemia. There is a consensus that FK506 when compared with CsA causes fewer changes in the blood pressure, serum lipids and renal function. Nevertheless, prolonged FK506 use is associated with a higher incidence of post-transplant diabetes mellitus (PTDM) [1–3]. Kidneys from FK506 treated patients can present arteriolar hyalinosis, tubular atrophy and interstitial fibrosis [4]. The major effect of CNIs on lymphocytes consisted of preventing IL-2 secretion by T helper cells that in turn impaired the immune response against the transplanted organ, opportunistic infections and tumor development. Lerut et al. reported on 20 liver transplanted patients treated with FK506 whose total infection rate during the first 3 months post-transplant was 80% and cancer development was 15% in a long-term follow-up over 5 years [5].

FTY720, a new synthetic analog of a compound in the extracts of *Isaria sincclairii*, has been described as a sphingosine-1-phosphate (S1P) receptor agonist. S1P binding to lymphocytes, endothelial and mesangial cells transmits signals through a family of G-protein-coupled receptors to control cellular differentiation and survival, as well as vital immune cell functions [6]. FTY720 has been used in experimental models of transplantation, ischemia–reperfusion injury, autoimmune diseases, and tumors [7–11]. FTY720 causes a dramatic decrease in peripheral blood lymphocytes (PBL) and reduces the number of infiltrating cells in graft tissues [12,13]. Although FTY720 enhances lymphocyte responses to homing chemokines resulting in sequestration of these cells in the lymph nodes, it is unlikely that lymphopenia would be the only mechanism correlated with its therapeutic effects in reducing acute rejection.

In contrast to classic immunosuppressive drugs, FTY720 does not inhibit proliferation or activation of T cells; therefore, it does not interfere with the immunity against systemic viral infections, which may reduce the incidence of opportunistic infections after transplant [14].

In renal transplanted patients, FTY720 presented the same efficacy as MMF (mofetil mycophenolate) in preventing acute rejection, without causing the common side effects of CNIs [15]. Budde et al. also demonstrated that side effects commonly related to CNI use, such as diabetes mellitus, nephrotoxicity, neurotoxicity, hepatotoxicity and myelosuppression, were not observed with FTY720 [16]. In animal models, the combination of FTY720 and FK506 resulted in increased graft survival in renal, cardiac and liver transplantation [17–19].

It has recently been shown that FTY720 (1 mg/kg/day) administered to rodents during 21 days did not cause renal functional or structural changes[20,21]. However, the possible side effects of FTY720 alone or in combination with CNIs require further investigation. Therefore, it was our aim to investigate whether FTY720 in combination with FK506 has any effect on parameters associated with renal function, development of diabetes or on changes in blood leukocyte numbers.

2. Material and methods

2.1. Animals

Eight to 10-week-old male C57BL/6 mice were used (bred in local colony). Mice were kept in collective cages except for the last day of the experiment period when they were placed in individual metabolic cages during 24 h for urine collection. In both periods they received a standard mice diet and water *ad libitum*. Animals were cared for in accordance with the Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) and the Brazilian Committee on Animal Experimentation.

2.2. Experimental groups

The control group mice were neither submitted to FTY720 nor FK506 administration and were followed up for 21 days. In FTY720, FK506, and FTY720+FK506 groups, animals received a daily dose of the designated treatment during 21 days.

One mg/kg FTY720 (Novartis, Basel, Switzerland) and/or FK506 2 mg/kg (Fujisawa, Japan), both diluted in sterile distilled water, were administered by gavage.

2.3. Metabolic cages

On the 21st day of drug administration mice were placed in metabolic cages (Nalgene-Mini Mitter Co., Inc. OR, USA) during 24 h for urine collection. Mice were then anesthetized by intraperitoneal injections of Xylazine (Agribrands, Brazil) and Ketamine (Vetbrands, Brazil) diluted in 10 mL of sterile PBS (phosphate buffered solution—OXOID LTD Hampshire England). Following anesthesia, animals were placed on a temperature controlled surgery table (Braile Biomédica, Brazil) and the abdomen was opened with the aim of collecting blood from the vena cava and kidney harvest. Blood and urine were used for the investigation of biochemical and cellular parameters whereas kidneys were removed for histology examination.

2.4. Biochemical measurements

Urine, collected from the metabolic cages, was centrifuged for 7 min at 1500 rpm and kept at −4 °C before analysis. Blood was harvested from the vena cava; 10 μL was used for a blood