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Comparison of long-term impact of immunosuppressants at therapeutic doses on hepatic function and histological changes in unilateral nephrectomized rats

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ABSTRACT

Cyclosporine, tacrolimus and sirolimus are commonly used in renal transplant recipients to prevent rejection. Various adverse effects of these agents on the multiple organ system have been reported clinically. However, animal studies are necessary to determine and compare these effects on individual organ given the presence of multiple confounding factors and multi-pharmacy in clinical settings. In a physiologically and clinically relevant rat model of unilateral nephrectomy, the long-term impacts of commonly used immunosuppressants at doses equivalent to the therapeutic levels used for post-renal transplant patients on hepatic function and histological changes of the liver were examined. Cyclosporine induced significant hepatocellular injury, impairment of synthetic function of the liver, hyperbilirubinemia and cholestasis, and dyslipidemia accompanied by profound histological changes of hepatic structures on both light and electron microscopic examinations. On the other hand, neither tacrolimus nor sirolimus developed any hepatotoxic effects except for more remarkable dyslipidemia was observed in animals treated with sirolimus. Our study indicates that long-term administration of commonly used immunosuppressants has various impacts on biochemical parameters as well as histological alterations of the liver even at therapeutic levels. These data may therefore provide useful information for judicious selection of immunosuppressive agents based on different clinical settings.

1. Introduction

Renal transplantation has become the treatment of choice for patients with end-stage renal disease (ESRD) worldwide due to its superior survival benefit and quality of life. According to a report from the Organ Procurement and Transplantation Network (OPTN, optn.transplant.hrsa.gov), there had been more than 350,000 renal transplants performed in the United State from 1988 to 2013. Cyclosporine, tacrolimus and sirolimus are most important components in commonly used immunosuppressive regimens, which

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have resulted in a dramatic improvement in the outcomes observed in renal transplant recipients over the past 30 years. Long-term administration of these medications, however, has contributed variably to the risks of cardiovascular disease, infection, malignancy, and nephrotoxicity, leading to the development of late mortality and chronic allograft dysfunction.

Hepatotoxicity including cholestasis, hyperbilirubinemia, elevated transaminases, inhibition of protein synthesis and disturbed lipid metabolism, has been reported in both human and experimental animals receiving cyclosporine (Lorber et al., 1987; Rezzani, 2004). However, information on the effects of tacrolimus and sirolimus on liver is limited. Animal studies are necessary to determine or compare specific effects of these agents on individual organ system given the presence of multiple confounding clinical factors and multi-pharmacy in renal transplant recipients. Unfortunately, available experimental studies addressing the adverse effects of immunosuppressants are generally conducted in animals with two kidneys, which is not the case in renal transplant recipients who have only one functional kidney. The majority of such studies were also relatively short-term with the use of higher toxic doses of drugs via parental routes. The present study was therefore designed to examine the impacts of long-term oral administration of commonly used immunosuppressants at therapeutic doses on biochemical parameters and histological alterations of the liver in rats subjected to unilateral nephrectomy.

2. Materials and methods

2.1. Materials

Male Sprague-Dawley rats (6 weeks old) weighting 160–180 g were provided by Guangdong Laboratory Animal Center. The animals were housed at constant temperature with a 12-h light/dark cycle and allowed free access to standard rodent chow and tap water according to the policy established by the International Council for Laboratory Animal Science. All test substances are obtained from Hangzhou Zhongmei Huadong Pharmaceutical Co., Ltd (Hangzhou, China).

2.2. Experimental design

All proposed animal studies were approved by the Institutional Animal Care and Use Committee. Forty rats were randomly divided into 5 experiment groups of eight animals each: A, control; B, unilateral nephrectomy alone (UN); C, UN plus cyclosporine; D, UN plus tacrolimus; E, UN plus sirolimus. Rat model of unilateral nephrectomy was performed as detailed in our previous study (Chen et al., 2013). One week after recovery from surgery, animals received daily treatment with either drinking water (Groups A and B) or one of the drugs diluted in water (Groups C–E) via oral gavage for 8 weeks. Since the first therapeutic doses of cyclosporine and tacrolimus in renal transplant recipients are 5 mg kg^{-1} day^{-1} and 0.15 mg kg^{-1} day^{-1} and the therapeutic dose for sirolimus is usually 2 mg/day, the doses of these agents to be administrated to the rats were 25 mg kg^{-1} day^{-1}, 0.8 mg kg^{-1} day^{-1}, and 0.2 mg kg^{-1} day^{-1} respectively according to the formula

$$DW_1 = \left[\frac{(R_1 W_2^{1/3})}{(R_2 W_1^{1/3})}\right] DW_2,$$

where DW is the drug dose, R is body index (0.09 for rat and 0.1 for human), and W is the standard weight (200 g for rat and 60 kg for human). The concentrations of drugs in blood were measured to confirm their levels in vivo (mean cyclosporine level is 1268.6 μmol/L and mean tacrolimus level is 1.53 μg/L respectively; we did not measure the levels of sirolimus). At the end of 8 weeks, blood was collected for the measurement of various biochemical parameters related to hepatic functions by routine laboratory assay. The liver was harvested and fixed in 10% formalin, embedded in paraffin, sectioned at 2 μm and stained with H&E (hematoxylin and eosin) by routine techniques. The histological analysis was then performed using a laboratory upright microscope and the morphological changes were examined by a pathologist who was blind to the sample subgroups. For evaluation of ultrastructural changes of the liver, tissue sections were fixed with 2.5% glutaraldehyde and 1% osmium tetroxide and embedded in epoxy resin. Ultra-thin sections (60–90 nm) were stained with 2% uranyl acetate and 2% lead citrate prior to examination under a transmission electron microscope.

2.3. Statistical analysis

Values were reported as means ± standard deviation. Statistical analysis was performed using a SPSS version 17.0 statistics program and the one-way analysis of variance + Post hoc LSD test was used to compare the difference between each group. A p value of less than 0.05 was used to determine the level of statistical significance.

3. Results

3.1. Body weight change

All animals survived to the end of 8 weeks, which is proportional to 5 human years (Quinn, 2005). As shown in Table 1, the general health of majority of the animals (Control, UN, UN+FK506, and UN+Rapa) remained fair as indicated by continuous weight gain during the entire study period. However, rats subjected to unilateral nephrectomy and received cyclosporine (UN+CsA) did not gain as much as the others (Table 1). Initially food consumption was reduced in these animals but later on they became subdued and irritable. Hair loss was clearly evidenced in these rats on the face and fore limbs.

3.2. Hepatocellular injury

Elevation of serum transaminases are important markers for liver injury. As shown in Fig. 1A and B, the levels of serum transaminases were essentially the same in UN rats compared with controls. AST (aspartate aminotransferase), however, was significantly increased in UN rats that received cyclosporine (UN+CsA). ALT (alanine aminotransferase) was also increased in these animals but did not reach statistical significance. By contrast, treatment with tacrolimus or sirolimus in UN rats (UN+FK506 or UN+Rapa) did not affect either AST or ALT (Fig. 1A and B). These results indicated that administration of cyclosporine but not tacrolimus or sirolimus induce hepatocellular injury as indicated by the elevation of transaminases in UN rats.

Table 1
Body weight change in unilateral nephrectomized rats treated with cyclosporine, tacrolimus or sirolimus.

Group	N	Body weight (g)		Body weight change (%)
		Before	After	
Control	8	181.61 ± 5.65	345.17 ± 15.31	158.75 ± 15.68
UN	8	180.75 ± 6.22	339.62 ± 11.97	160.39 ± 14.75
UN+CsA	8	183.05 ± 7.13	296.50 ± 22.69	112.24 ± 0.20.16[*,#,^]
UN+FK506	8	180.51 ± 8.95	335.30 ± 17.51	154.78 ± 11.32
UN+Rapa	8	182.28 ± 8.65	342.56 ± 15.29	160.91 ± 13.51

UN, unilateral nephrectomy; CsA, cyclosporine; FK506, tacrolimus; Rapa, sirolimus.
[*] $p < 0.05$ compared with UN group.
[#] $p < 0.05$ compared with UN+FK506 group.
[^] $p < 0.05$ compared with UN+Rapa group.