SYNERGISTIC EFFECT OF RAPAMYCIN AND CYCLOSPORINE IN PREVENTION OF ACUTE KIDNEY ALLOGRAFT REJECTION IN THE MOUSE

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The effect of rapamycin (RAPA) and cyclosporine A (CsA) monotherapy and combination therapy was examined in prevention of kidney allograft rejection in the mouse. Both drugs were administered orally for up to 14 days in BALB/c (H-2^d) to C57BL/6 (H-2^b) mice strong combination. Six groups were treated with RAPA and/or CsA. This study shows that concomitant therapy of RAPA and CsA produces strong synergistic interaction in prolonging renal allograft survival in mice when compared with monotherapy of RAPA or CsA.

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Results from preclinical studies predict that use of selected combinations of immunosuppressants in patients will be more efficacious with less side effects. Rapamycin (RAPA), a hydrophobic 31-member macrocyclic lactone ($C_{51}H_{79}NO_{13}$; molecular mass 914.2 daltons) produced by *Streptomyces hydroscopicus*,[1–3] inhibits the development of a variety of experimental autoimmune diseases in mice. It also prolongs allograft survival in mice, rats, pigs, dogs, and primates.[4–6] RAPA has a strong antiproliferative effect on T cells stimulated via the T-cell receptor/CD3 pathway. We have reported previously that RAPA also acts directly on B cells and effectively inhibits Ig production in vitro and vivo.[7,8]

In vitro, cyclosporine (CsA) and RAPA synergistically inhibit [^{3}H] thymidine incorporation by human peripheral blood leukocytes upon stimulation with concanavalin A, phytohemagglutinin, or alloantigens in mixed lymphocyte culture.[9] Combined therapy of RAPA and CsA produced synergistic interaction in prolongation of cardiac allograft survival in mice, heart, small bowel, pancreas as well as corneal allograft survival in rats.[10–14] In contradistinction to rats, mice are relatively resistant to CsA and other immunosuppressants,[10] a feature that may resemble patients who display a relative resistance to CsA immunosuppression.[15] The mouse transplantation model offers more advantages for immunological research. In this study, we evaluate the combined effect of RAPA and CsA in prevention of acute renal allogtraft rejection in mice.

MATERIALS AND METHODS

Animals

Male inbred BALB/c (H-2^d) and C57BL/6 (H-2^b) mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Mice weighed between 22 and 25 g. They were housed in controlled light/dark cycles and allowed free access to water and mice chow.

Drugs

Oral formula of RAPA was donated by Wyeth-Ayerst Research (Rouses Point, NY). CsA was obtained as 100 mg/ml oral solution from Novartis Pharma Canada (Doval, Canada). Final CsA doses diluted in pure olive oil according to recipient's weight were administered daily by gavage for 14 days.

Kidney Transplantation

Donor and recipient mice were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg). Kidney transplantation was performed by a method described by Zhong et al.[16] with some modifications. Briefly, the donor's left kidney was perfused through the aorta with 4°C heparinized, lactated Ringer's solution. It was harvested en bloc with the left ureter together with a small, elliptical patch of bladder, a segment of aorta containing the left renal artery, and the left renal vein close to the inferior vena cava (IVC). The kidney was preserved in the same solution for

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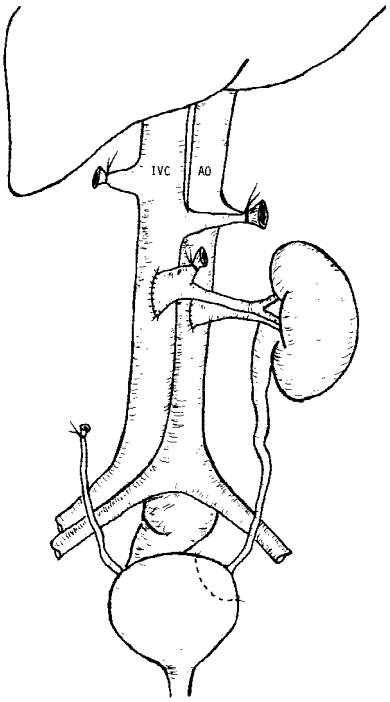

Figure 1. Completed recipient kidney transplantation in the mouse (AO, aorta; IVC, inferior vena cava).

less than 30 min. The recipient mouse was anesthetized and the left nephrectomy was performed after transecting the ureter, renal artery, and vein. End-to-side anastomoses were done between the donor renal vein and recipient IVC and between the donor aortic segment and recipient aorta with 11-0 nylon sutures (Shapoint, Reading, PA). A patch of donor bladder to recipient bladder anastomosis was completed by continuous 10-0 sutures (Fig. 1). Before closing the abdomen, the naive right kidney of the recipient was removed. The time of rejection was defined as the last day before death of the recipients. Animals that died within 3 days after transplantation (less than 20%) were excluded from the analysis.

DESIGN OF EXPERIMENTS

C57BL/6 recipient mice received BALB/c kidney grafts and were treated with CsA and/or RAPA daily at different doses by gavage from day 1 posttransplantation for 14 days. Six groups ($n \geq 6$) were involved in this study (Table 1).

DATA ANALYSIS

Mice kidney allograft survival times are presented as a mean survival time (MST ± SD), with comparison among groups performed by Gehan's survival test. The median-effect principle[17–19] is based on the premise that the effect of each agent is related to its dose and, therefore, can be calculated using the following equation:

$$(fa/fu) = (D/Dm)^m \quad (1)$$

where fa and fu represent the fractions of the system that are affected (percent inhibition or rather days of survival beyond controls) and unaffected (1-fa), respectively, by the drug at dose D. Full protection ($fa = 1$) is defined as at least a 60-day allograft survival. Dm is the dose required for 50% inhibition (ED_{50}), the median effect; m is a coefficient that describes the sigmoidicity of the dose-effect curve. The interaction between the two drugs, synergism, summation, or antagonism is assessed by the combination index (CI):

$$CI_x = \frac{D_1 \text{ combined}}{D_1 \text{ alone}} + \frac{D_2 \text{ combined}}{D_2 \text{ alone}} + \frac{(D_1 \text{ combined})(D_2 \text{ combined})}{(D_1 \text{ alone})(D_2 \text{ alone})} \quad (2)$$

Where D_1 combined and D_2 combined represent the amount of drug 1 and drug 2, respectively. D_1 and D_2 each display × inhibition for the mutually exclusive case where both drugs have different modes of action. According to Chou's interpretation, CI values less than 1.0 (<1) suggest synergism, whereas those equal to 1.0 ($= 1$) indicate summation, and those above 1.0 (>1) show antagonism. A computer software program was used to assess the dose-effect parameters (D_m, m, and r) and CI values (Calcusyn, Biosoft, MO, USA).

Table 1. Effect of RAPA and CsA in Prevention of Acute Renal Allograft Rejection in Mice

RAPA/CsA (mg/kg)[a]	Survival (MST ± SD)[b]	CI value[c]	*P* value*
G1			
—/—	7.4 ± 0.8		
G2			
2.0/—	16.8 ± 3.9		0.0001
G3			
4.0/—	37.9 ± 3.7		0.0001
G4			
—/2.0	25.0 ± 2.9		0.0001
G5			
—/4.0	37.5 ± 6.6		0.0001
G6			
2.0/2.0	40.2 ± 3.2	0.030	0.0001

[a]RAPA and CsA were administered by gavage for 14 days from day 1 after transplantation.
[b]Arbitrarily evaluated to a maximum of 60 days, $n \geq 6$ in each group.
[c]CI value for RAPA and CsA interaction calculated as shown in "Data Analysis."
**Relative to G1.*

RESULTS

Untreated C57BL/6 recipient mice received BALB/c kidney allograft and an MST of 7.4 ± 0.8 days was observed. In contrast, C57BL/6 recipients treated with RAPA

2.0 or 4.0 mg/kg/day orally for up to 14 days had a significantly prolonged BALB/c kidney allograft survival with an MST of 16.8 ± 3.9 and 37.9 ± 3.7 days, respectively compared with naive controls ($P = 0.0001$). Meanwhile, C57BL/6 recipients treated with CsA 2.0 or 4.0 mg/kg/day orally for up to 14 days also significantly prolonged BALB/c kidney allograft survival with an MST of 25.0 ± 2.9 and 37.5 ± 6.6 days, respectively ($P = 0.0001$). Furthermore, concomitant use of a low dose RAPA 2 mg/kg/day with a low dose of CsA 2 mg/kg/day produced a very strong synergistic interaction when compared with single RAPA or CsA use only (40.2 ± 3.2 days; CI = 0.030, Table 1).

DISCUSSION

This study examined the effects of RAPA and CsA monotherapy and combination therapy in prolongation of kidney allograft survival in the mouse. RAPA or CsA alone significantly prolonged renal allograft survival in a dose-dependent fashion. However, RAPA alone can induce significant side effects in several nonrodent species, including primates and pigs,[6,20] and has been shown to be particularly toxic in dogs.[21,22] The toxic effects in dogs induced with RAPA at a dose as low as 0.25 mg/kg/day included fever, anorexia, vomiting, severe weight loss, gastrointestinal ulceration and vasculitis, extreme lethargy, and death. Ochiai et al.[21] reported that CsA at a dose of 2.5 mg/kg/day both reduced RAPA's toxic effects in dogs and improved renal allograft survival. Hartner et al.[23] reported that the combined use of low-dose RAPA, antilymphocyte serum (ALS), and CsA could induce long-term renal graft survival in dogs without serious RAPA-induced side effects. Drug combination therapy permitted reduction of immunosuppressive drug doses from 10- to 200-fold and optimized efficacy while minimizing toxicity.[9–11] Both RAPA and CsA are potent immunosuppressants but with different side effects. RAPA-associated toxicities include thrombocytopenia, leukopenia, and an increase in cholesterol and triglyceride levels.[24] CsA-induced side effects include nephrotoxicity, neurotoxicity, and hyperglycemia.[25]

The potential benefits of the combination therapy of RAPA and CsA may include the prevention of allograft rejection in clinic organ transplantation. Because both RAPA and CsA are highly bioavailable as oral preparations, an outpatient antirejection therapy may be initiated and the cost of hospitalization, morbidity, and risks associated with parenteral antirejection agents are avoided. Furthermore, the potentially lethal infection complications and myelosuppression secondary to high-dose corticosteroids and antilymphocyte agents are expected to be minimum.

Different organs, strains, or species can yield different results in experimental organ transplantation. In the rat model, a very low dose of RAPA (0.8 mg/kg) can prevent heart, kidney, pancreas, and small bowel allograft rejection[26,27]; this has not been the case in the mouse model. In this study, as in our previous study of small bowel transplantation in mice,[28] RAPA 4 mg/kg/day orally for 14 days significantly prolonged kidney or small bowel allograft survival. Thus, mice are resistant to the immunosuppressive effects of RAPA; similar observations were made for CsA.[10] Another difference is that in the rat model, RAPA is more effective in the prevention of allograft rejection with 10- to 100-times stronger effect than CsA.[4,5,26,27] This was not the case in the present study in the mouse model. Equivalent doses of RAPA and CsA (4 mg/kg/day × 14 days) prolonged renal survival without statistically significant difference ($P = 0.869$). This phenomenon was repeatable in our mouse small bowel allografting model with same treatment.[28]

In conclusion, this study documented that concomitant therapy of low doses of RAPA with CsA produces synergistic effects in prevention of kidney allograft rejection in the mouse. This result warrants further investigation of the combination of RAPA and CsA in preclinical and clinical organ transplantation.

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