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ASP0028 in combination with suboptimal-dose of tacrolimus in *Cynomolgus* monkey renal transplantation model

Hao Dun [a], Lijun Song [a], Anlun Ma [a], Yanxin Hu [a], Lin Zeng [b], Jieying Bai [b], Guangzhou Zhang [b], Liangyan Zhang [b], Kumi Koide [c], Yohei Okada [c], Kaori Hanaoka [c], Rie Yamamoto [c], Jun Hirose [c], Tatsuaki Morokata [c], Pierre Daloze [a], Huifang Chen [a,*]

[a] Department of Surgery, Research Center, CHUM, Notre-Dame Hospital, University of Montreal, Montreal, Canada
[b] Laboratory Animals Center, the Academy of Military Medical Sciences, Beijing, China
[c] Drug Discovery Research, Astellas Pharma Inc., Japan

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ABSTRACT

FTY720, a S1P-receptor modulator, has shown to be effective in several transplant and autoimmune disease models, via modulating lymphocyte homing into secondary lymphoid organs (SLOs), and thereby reducing these cells in peripheral blood. ASP0028, a newly developed $S1P_1/S1P_5$-selective agonist, presented comparable efficacy to FTY720 and wider safety margins than FTY720. In this study, we assessed the efficacy and safety of ASP0028 co-administered with suboptimal-dose of tacrolimus in the *Cynomolgus* monkey renal transplantation model. Seven animals in group-1 or group-2 received mono-tacrolimus 1.0 mg/kg once a day (QD), or ASP0028 0.6 mg/kg plus tacrolimus 1.0 mg/kg QD, respectively. Eight animals in group-3 received ASP0028 1.2 mg/kg plus tacrolimus 1.0 mg/kg QD. The allograft median survival time (MST) in group-2 and group-3 were significantly extended to 41 and 61.5 days, versus that of 28 days in group-1 ($p = 0.036$ and 0.001, respectively). ASP0028 administration remarkably reduced absolute numbers of peripheral lymphocytes, particularly subsets of $CD4^+$/ or $CD8^+$/naive and central memory cells, $CD4^+$/Treg cells, and to a lesser extent on B cells, but not $CD4^+$/ or $CD8^+$/ effector memory cells and NK cells. These data show ASP0028 combined with suboptimal-dose of tacrolimus effectively prolongs renal allograft survival in nonhuman primates (NHPs) with well tolerated safety, supporting its further investigation to optimize CNI-sparing regimens.

1. Introduction

S1P is a sphingolipid mediator, which regulates multiple critical cellular processes through binding to five G protein-coupled S1P receptors ($S1P_{1-5}$) [1,2]. $S1P_1$ is dominantly expressed on lymphocytes and thymocytes, and regulates lymphocyte egress from SLOs and thymus [3]. FTY720 is an agonist at four of the five S1P receptors, including $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$, but not $S1P_2$ (4). After phosphorylation, FTY720 binding $S1P_1$, causes internalization of receptors and consequently inhibits $S1P/S1P_1$ recipient-dependent lymphocyte egress from SLOs. As a result, lymphocytes are sequestered in SLOs and thereby fail to recirculate into the peripheral blood and subsequently reach target tissues [5]. Because of these features, $S1P_1$ appears to be a critical regulator of lymphocyte trafficking, and therefore becomes a key target for development of immunosuppressive agents.

FTY720 has been proven to significantly decrease the number of circulating T cells on selected lymphocyte subsets and B cells [6–9]. Based on this mechanism, FTY720 provided a variety of effects on autoimmune [10–12] and transplant models [13–17]. Moreover, due to distinct mechanisms of immunosuppressive action, FTY720 showed a synergistic effect on allograft survival when combined with cyclosporine or sirolimus in rodent and NHP models [15–17]. In September 2010, FTY720 was approved by the FDA for treatment relapsing multiple sclerosis (MS) [18]. In addition, two phase-3 clinical trials for renal transplant had been performed to evaluate the efficacy and safety of FTY720 in combination with suboptimal-dose of cyclosporine [19,20]. However, the trials failed to meet the endpoints, and therefore further study of the use of FTY720 was discontinued in this indication [2]. Of note, in all clinical trials of FTY720, bradycardia is one of the most commonly reported side effects [21–23]. This bradycardia is presumably because of agonistic activity of FTY720 toward $S1P_3$, since FTY720 does not cause bradycardia in $S1P_3$ knockout mice [24]. Based on these observations, $S1P_1$-selective compound is presumed to be devoid of side-effects,

Abbreviations: $AUC_{(0-24)}$, the area under the plasma concentration-time curves from 0 to 24 h; C_{max}, the maximum plasma concentration; CNI, calcineurin inhibitor; MS, multiple sclerosis; MST, median survival time; NHPs, nonhuman primates; PD, pharmacodynamic; PK, pharmacokinetic; QD, once daily; S1P, sphingosine-1-phosphate; SCr, serum creatinine; SLOs, secondary lymphoid organs; T_{max}, the time to reach C_{max}.

* Corresponding author.
E-mail address: hui.fang.chen@umontreal.ca (H. Chen).

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described in FTY720 clinical studies, e.g. bradycardia and macular edema.

ASP0028 is a newly developed $S1P_1/S1P_5$-selective agonist in Astellas Pharma Inc. Unlike FTY720, in vitro much higher concentration of ASP0028 is required to stimulate $S1P_3$ (unpublished data). The preliminary study showed that ASP0028 at 1.0 mg/kg level, similar to FTY720 at 0.1 mg/kg, significantly decreased the number of peripheral lymphocytes in rats. In addition, heart transplant studies in rats indicated that ASP0028 combined with suboptimal-dose of tacrolimus significantly prolonged allograft survival, comparable to that of FTY720 in combination with suboptimal-dose of tacrolimus (unpublished data). More importantly, our preliminary study showed that ASP0028 has a wider margin of safety than FTY720, in terms of side-effects of macular edema and bradycardia.

Combination therapies of different immunosuppressive agents with distinct mechanisms of action are a valid strategy for transplant patients to minimize dose-related toxicities. In this *Cynomolgus* monkey renal transplantation model, tacrolimus at suboptimal-doses of 1.0 mg/kg was suggested in a tacrolimus-based combination therapy for testing a new compound [25]. Accordingly, in the present study, we evaluated the efficacy and safety of ASP0028 0.6 or 1.2 mg/kg in combination with tacrolimus 1.0 mg/kg in NHP renal transplantation model. Moreover, we investigated whether there is a differential inhibitory effect of ASP0028 on certain subsets of peripheral lymphocytes. In addition, we analyzed the pharmacokinetic (PK) profile of ASP0028 in combination with suboptimal-dose of tacrolimus. We assume that the selectivity of ASP0028 in NHPs is comparable in humans. Therefore, findings obtained from this study will contribute to a better understanding of the mechanism of action of ASP0028, which is a key for its further development as a candidate drug for optimizing CNI-sparing regimens in clinical transplant.

2. Materials and methods

2.1. Animals

Twenty-two male *Cynomolgus* monkeys (*Macaca fascicularis*), aged 3–6 years, with body weight of 3.2–5.2 (4.1 ± 0.42) kg, and hepatitis B virus, hepatitis C virus, simian immunodeficiency virus, simian varicella virus, and herpes B virus free, were obtained from Laboratory Animals Center of the Academy of Military Medical Sciences (AMMS), Beijing, China. The experimental protocol was approved by the Ethical Committee for Animal Experimentation at Laboratory Animals Center of the AMMS, and all procedures were performed according to the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Office of Animal Care and Use. Before study entry, all animals were quarantined for two weeks with general health screening. Each animal with a unique identification number was randomly assigned to an experimental group. All animals were housed in individual cages and allowed free access to water, monkey chows and fruits.

2.2. Life supporting kidney transplantation

In the present study, each animal served as both donor and recipient. Donor and recipient monkey pairs were selected by ABO blood-type compatibility, and the stimulation index of mixed lymphocyte reaction (MLR) ≥ 2.5. Life supporting kidney transplantation was performed as described previously [26,27]. Briefly, left kidneys were harvested *en bloc* and exchanged between paired animals for transplant. The allograft then was implanted into recipient abdomen by end-to-side anastomoses of renal artery to aorta and renal vein to vena cava, and by end-to-end anastomosis of donor and recipient ureters. After grafting, the right native nephrectomy was immediately performed.

2.3. Experimental design and treatment protocols

Animals were randomly divided into three experimental groups for a maximum of 90-day observation as shown in Table 1. Based on an earlier experiment [25], the suboptimal-dose of tacrolimus 1.0 mg/kg was chosen as a control. Accordingly, Animals in group-1 ($n = 7$) received mono-tacrolimus 1.0 mg/kg QD, in group-2 ($n = 7$) received ASP0028 0.6 mg/kg plus tacrolimus 1.0 mg/kg QD, and in group-3 ($n = 8$) received ASP0028 1.2 mg/kg plus tacrolimus 1.0 mg/kg QD, respectively. Tacrolimus (lot No. 701732K), manufactured by Astellas Pharma Inc., was orally given on day 0 (the day of kidney transplant) immediately after kidney transplantation until day 90, whereas ASP0028 was orally administered from 2 days before surgery (day -2) till day 90.

2.4. Biochemical and hematologic determinations

Serum creatinine (SCr) levels were intensively monitored on day -7 and post-operative day 1, 3, 5 and 7 during the first week, and then at least twice per week for the first month, and thereafter weekly. The others were determined as described in our previous study [26].

2.5. Lymphocyte subsets

Lymphocyte phenotyping was performed on a subset of 4 animals of each group (Table 1). Blood samples were collected on day -7 and post-operative day 0, 7, 14, 28, 56 and 84 before dosing to determine the effects of ASP0028 on certain lymphocyte subsets. Lymphocyte subsets, including $CD3^+$ (absolute number T cells), $CD3^+/CD4^+$ (T helper cells), $CD4^+$-$CD28^+CD95^-$ ($CD4^+$ naïve cells), $CD4^+CD28^+CD95^+$ ($CD4^+$ central memory cells), $CD4^+CD28^-CD95^+$($CD4^+$ effector memory cells), $CD3^+/CD8^+$ (T suppressor/killer cells), $CD8^+CD28^+CD95^-$ ($CD8^+$ naïve cells), $CD8^+CD28^+CD95^+$ ($CD8^+$ central memory cells), $CD8^+$-$CD28^-CD95^+$ ($CD8^+$ effector memory cells), $CD3^+CD4^+CD25^+CD127^-$ ($CD4^+$ Treg cells), $CD3^-CD20^+$ (B cells) and $CD3^-CD16^+$ (NK cells) were analyzed on the FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) using the Becton Dickinson software CellQuest Pro.

2.6. Pharmacokinetic evaluations

PK analyses for blood tacrolimus in all animals, and for plasma ASP0028 in animals of group-2 and -3 were conducted by a validated LC-Tandem Mass Spectrometry (LC/MS/MS) system, using Analyst Data Acquisition (Version 1.4.2) and Watson 7.3 LIMS software. The blood concentrations of tacrolimus were measured before and after 1 h administration on day 14, 28, 56 and 84. The plasma concentrations of ASP0028 were monitored before and after 4 h administration on day 0 and 7. Moreover, additional PK parameters of ASP0028 were evaluated on day 14, 28, 56 and 84. The C_{max} of ASP0028 was achieved from observation of concentrations of trough (0), 2, 4, 8, 12 and 24 h after administration, and the T_{max} was determined from above time points. In addition, $AUC_{(0-24)}$ for ASP0028 was analyzed.

2.7. Histopathologic determinations

All recipients underwent complete gross necropsies and histopathologic examinations as described in our previous study [26]. The presence and degree of renal allograft rejection was scored according to the Banff '97 criteria of renal allograft pathology [28].

2.8. Statistical analysis

All data were described as mean ± standard deviation (SD). Analyses of statistical differences among groups were performed using the two-tailed *t*-test. Allograft survival times presented as MST were compared among groups by log-rank test. Statistical analyses were conducted using SPSS 13. A p value of <0.05 was considered statistically significant.