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Letter to the Editor

Pharmacokinetics of Oral Mycophenolate Mofetil in Dog: Bioavailability Studies and the Impact of Antibiotic Therapy

An improved understanding of the pharmacokinetics of mycophenolic acid (MPA), the active metabolite of the immunosuppressant mycophenolate mofetil (MMF), is needed because of the increasing use of MMF in patients undergoing solid-organ transplantation [1] or hematopoietic cell transplantation (HCT) [2-4]. Plasma concentrations of MPA have been correlated with various clinical outcomes in solid-organ transplant recipients [1]. However, only recently have pharmacodynamic associations been described in patients undergoing HCT, specifically the correlation of increased MPA concentrations at steady state [3] and the unbound MPA area under the concentration-time curve (AUC) [4], with higher degrees of donor T cell chimerism [3] and lower incidences of graft rejection [3,4], respectively.

Recent studies in a well-established preclinical canine model of HCT have identified effective immunosuppressive drug combinations that include MMF for the prevention of acute allograft rejection and graft-versus-host disease [5,6]. In these studies, MMF was administered subcutaneously at a dose of 10 mg/kg twice daily. We sought to determine in a healthy 25-month-old male beagle the bioavailability and interdose variability of oral MMF to develop an understanding of drug dosing required for substituting oral for subcutaneous MMF. Because enterohepatic circulation mediated through gram-negative intestinal bacteria plays a major role in MPA pharmacokinetics [7], we also addressed the question of whether gut decontamination with nonabsorbable antibiotics affects the enterohepatic circulation in the dog, which could have potential clinical implications for canine marrow graft recipients. MPA bioavailability was investigated on 3 occasions, each separated from the others by 1 month, after single doses (ie, 10, 15, and 20 mg/kg) of oral MMF (CellCept Oral Suspension, Roche Laboratories. Nutley, NJ; 200 mg/mL) and compared with that of a single subcutaneous dose of 10 mg/kg MMF (CellCept Intravenous, Roche Laboratories; 25 mg/mL); each oral dose was separated from the baseline subcutaneous dose by a 3-day rest period. The effect of nonabsorbable antibiotics on oral MMF pharmacokinetics was evaluated on 4 occasions, 1 month apart. At baseline, the dog was given a single dose of 15 mg/kg oral MMF, followed by 3 days of rest, and then received a nonabsorbable antibiotic mixture consisting of a 1:1 ratio of neomycin sulfate (Biosol, The Upjohn Company, Kalamazoo, MI) and polymyxin B sulfate (Polymyxin B, Bedford Laboratories, Bedford, OH), each administered at a dose of 1 g orally per total body weight, 3 times daily, 10 days before and concomitant with another dose of 15 mg/kg oral MMF. Plasma samples were obtained before and 1, 2, 4, 6, 8, 10, and 12 hours, respectively, after each MMF dose. Pharmacokinetic parameters were quantified and analyzed as previously reported [3]. The interdose variability was assessed by comparing the variances of the various measurements of the pharmacokinetic parameters using the F test; $P < 0.05$ was considered statistically significant.

The pharmacokinetic data are presented in Table 1. Bioavailabilities were 54%, 65%, and 87% after 10, 15, and 20 mg/kg oral MMF, respectively. The plasma concentration-time profile after oral MMF was characterized by secondary maxima induced by the enterohepatic circulation within 4-12 hours of dosing. There were statistically significant differences in interdose variabilities in the MPA clearance and total MPA maximum plasma concentration (C_{max}; $P = .02$ and .006, respectively), but not in the MPA-free fraction and AUC from 0 to 12 hours ($AUC_{0\text{-}12h}$; $P = .16$ and .07, respectively) after oral versus subcutaneous MMF administrations (Table 1). Administration of preceding and concomitant nonabsorbable antibiotics with a single oral MMF dose did not appear to reduce the MPA-free fraction, $AUC_{0\text{-}12h}$, clearance, and C_{max} when compared with oral MMF given without antibiotics (Table 1); however, the average of the secondary MPA peaks to the $AUC_{0\text{-}12h}$ was reduced by 17.2% ($P = .64$). The interdose variabilities in the MPA-free fraction, $AUC_{0\text{-}12h}$, clearance, and C_{max} were not statistically significantly different after oral MMF given with or without nonabsorbable antibiotics ($P = .30$, .39, .42, and .21, respectively; Table 1); high interdose variabilities, although not statistically significantly different, were seen in the amplitude of the

Table 1. *Pharmacokinetic Parameters in a Healthy Dog Following Administration of Single Doses of Subcutaneous and Oral MMF*

MMF Route/Dose (mg/kg)	*N*	Unbound MPA (%)	MPA $AUC_{0\text{-}12h}$ (mg*hr/ml)	MPA AUC Secondary Peak (%)	MPA Clearance (l/hr*kg)	MPA C_{max} (μg/ml)
SC						
10	3	2.09 ± 0.4*	20.89 ± 2.97*	NA	0.485 ± 0.064*	5.38 ± 0.7*
PO						
10	1	1.93	13.17	48.4	0.759	4.66
15	5	1.95 ± 0.23*	20.77 ± 10.8*	39.3 ± 11.66*	0.859 ± 0.390*	9.87 ± 9.15*
20	1	1.82	33.7	33.7	0.593	17.95
PO + nAb						
15	4	1.75 ± 0.16*	21.94 ± 12.4*	32.57 ± 28.81*	0.874 ± 0.502*	10 ± 5.48*

Abbreviations: $AUC_{0\text{-}12h}$ = area under the concentration-time curve from 0 to 12 hours; C_{max} = maximum plasma concentration; MMF = mycophenolate mofetil; MPA = mycophenolic acid; *N* = number; NA = not applicable; nAb = non-absorbable antibiotics.
*Average ± standard deviation.

secondary MPA peaks to the $AUC_{0\text{-}12h}$ after oral MMF given with preceding and concomitant nonabsorbable antibiotics compared with oral MMF given alone ($P = .06$; Table 1).

Our data suggested that interdose variability after oral MMF administration occurred even under well-controlled environmental conditions. Using oral MMF after HCT would expose the dogs to unwarranted risks due to excessive or suboptimal MPA concentrations that could lessen the potency of immunosuppression necessary for successful HCT; therefore, oral MMF would not be a good substitute for subcutaneous MMF in the preclinical canine HCT model, where MPA plasma levels are not routinely monitored and adequate levels of immunosuppression are essential for preclinical validation of the HCT protocols. Our findings support the need for rational individual therapeutic monitoring of MPA levels in patients undergoing HCT, where the use of pretransplantation test doses did not accurately predict total or unbound MPA AUC after HCT [4].

On average, the effects of nonabsorbable antibiotics on MPA pharmacokinetics were not significant relative to the interdose variability seen. It has been recently reported that patients after HCT had lower plasma MPA levels and a shorter MPA half-life after oral MMF administration compared with healthy volunteers or solid-organ transplant recipients [2]. That might be attributed to the decreased bacterial flora in the gastrointestinal tract from broad-spectrum prophylactic antibiotic use. Further, it has been suggested that a significant fraction of the interdose variability in MPA pharmacokinetics was caused by day-to-day fluctuations in the contribution of MPA enterohepatic circulation to the MPA AUC [8]. In addition to generating a hypothesis, our study provided preliminary data regarding the effects of nonabsorbable antibiotics on the enterohepatic circulation of MPA, which could be further investigated in patients after HCT.

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