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Short-term Pharmacokinetic Study of Mycophenolate Mofetil in Neonatal Swine

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ABSTRACT

Background. Mycophenolate mofetil (MMF) is an effective immunosuppressive agent that has been frequently used in laboratory animals including swine; however, the pharmacokinetic properties of MMF in swine have not been studied. This short-term study was designed to evaluate the feasibility and the pharmacokinetic profiles of MMF therapy in neonatal swine.

Materials and Methods. Twelve neonatal pigs were randomized into four groups including one control and three treated groups with oral MMF administered at 0.5, 1, and 2 g/m²/d for 4 days, divided by 2 half-doses at 9:00 and 17:00 (except day 4 during which MMF was not administered at 17:00). Blood samples were collected at 9:00 on days 0, 2, 3 and 4 for complete blood count and hepatic/renal function examination; the trough concentration of plasma mycophenolic acid (MPA) was also determined. On days 2 and 4, blood was collected to determine the area under the curve (AUC) of plasma MPA concentration. Animal body-weight growth and manifestations of MMF side-effects such as anorexia, vomiting, and diarrhea were also observed.

Results. MMF has no acute hepatic/renal toxicity in newborn pigs; however, less body-weight growth was observed in treated groups. In the control group, a spontaneous increase of lymphocyte count was observed; in contrast, MMF therapy with doses of 1 and 2 g/m²/d reduced both lymphocyte and monocyte counts of piglets. Oral MMF had high bioavailability in neonatal swine. MPA-AUC_{0-12h} of doses 0.5, 1, and 2 g/m²/d was 22.00 ± 3.32, 57.57 ± 34.30, and 140.00 ± 19.70 µg × h/mL, respectively. Neither MPA trough concentration (MPA-C₀), nor MPA maximum concentration (MPA-C_{max}) or MPA-AUC_{0-6h} had high correlation with MMF-dose. For surveillance of MPA exposure, MPA-C₀ had significant correlation with MPA-AUC_{0-12h} (Spearman's $\rho = 0.933$, $AUC_{0-12h} = 17.882 \times C_0 + 14.479$, $r^2 = 0.966$).

Conclusion. To reach adequate drug exposure and to reduce dose-dependent side effects, an MMF dose of 1 g/m²/d is recommended to be used as an initial dose for immunosuppressive therapy in piglets, and MPA-C₀ monitoring is the most practical strategy for experimental transplantation study.

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MYCOPHENOLATE MOFETIL (MMF) is a more recent immunosuppressive drug which does not interfere with the actions of calcineurin and will not cause nephrotoxicity [1–3]. As an ester prodrug of the active immunosuppressant mycophenolic acid (MPA), MMF is metabolized mainly by liver and intestinal esterases [4].

MPA is a noncompetitive, selective, and reversible inhibitor of inosine monophosphate dehydrogenase which is an important enzyme in guanosine nucleotide de novo biosynthesis. Because proliferation of T and B lymphocytes is critically dependent on de novo synthesis of purines, whereas other cell types can recover purines by using salvage pathways, MPA has specific cytostatic effects on lymphocytes. MPA can inhibit proliferation responses of T and B lymphocytes to both mitogenic and allogeneic stimulation [5]. MPA has no effect on the production or release of the cytokines (interleukins [ILs] 1 and 2) associated with early T cell signal transduction, so it is not effective in the treatment of ongoing acute rejection [6].

MMF is now frequently used in transplant recipients as an adjuvant immunosuppressant which effectively helps to reduce the administration of calcineurin inhibitors (CNIs) and relevant nephrotoxicity [7]. A decreased rate of acute rejection was reported for adult and pediatric renal transplantation patients who achieved an MPA exposure value, expressed by area under the plasma MPA concentration-time curve from 0 to 12 hours (MPA-AUC₀₋₁₂) after MMF administration, greater than 30 mg·h/L [8,9]. Clinical studies have also shown that the pharmacokinetic parameters of MPA exhibit wide inter- and inpatient variability, and that MMF doses are not correlated with MPA plasma concentrations in each patient [10].

The swine is an excellent model for a transplantation immunology research program because of its similarities to human on genetics and body size. MMF has been used in porcine experimental transplantation studies [11–15]. Representatively, in the studies of vascularized composite allotransplantation (VCA) in juvenile pigs [11,12], MMF was administered at a dose of 500 mg/d, with concomitant cyclosporine (CsA, 40 mg/kg/d) or tacrolimus (FK506, 1.5 mg/kg/d). These combinations of immunosuppressants have successfully prevented rejection to VCA. However, pharmacokinetic profiles of MMF in swine have never been defined in these studies.

To develop a preclinical model for study of tolerance induction in neonates and very young infants, we have previously established a vascularized composite tissue allograft model in newborn pigs [16]. We subsequently studied the pharmacokinetics and acute nephrotoxicity of oral CsA in newborn pigs (article in press, *Immunopharmacology and Immunotoxicology*). This study was designed to characterize MMF pharmacokinetic profiles, side-effects, as well as immunosuppressive effects on homeostasis-driven proliferation of lymphocytes in newborn pigs to choose a proper initial dose and a practical monitoring strategy for MMF in a neonatal swine model.

MATERIALS AND METHODS

Animals

Twelve neonatal domestic piglets with a patrilineal pedigree of Large White and a matrilineal pedigree of Youli, all of approximately 5- to 6-days old, with weights ranging from 1.22 to 2.16 kg, were housed together with the sow in one cage under the standard procedure for neonatal pigs in the Institute Claude Bourgelat animal research center of the national veterinary school of Lyon (VetAgro Sup-Campus Vétérinaire de Lyon). The sow was fed with a standard pig diet. Newborn piglets were breastfed exclusively and unrestrictedly. All animal experimentations were approved by the Ethical Committee of the Veterinary Campus of Lyon (Number – 2013/1322) and conformed to the European Guideline 2010/63/EU, for the care and use of animals for scientific purposes.

MMF Administration and Clinical Observation

MMF (CellCept®, 1 g/5 mL, oral suspension) was purchased from Hoffmann-La Roche Ltd. (Basel, Switzerland). Piglets were divided randomly into four groups with respect to the weight balance among groups; there were three piglets per group. In the control group (group 0), piglets were administered saline solution. In treated groups (group 1, 2, and 3), different doses of MMF (0.5, 1, and 2 g/m²/d, respectively) were administered. During days 0–4, piglets were weighed every morning at 8:30, and then body surface area was calculated according to body weight by a specific formula from DeRoth et al [17]. MMF was administered orally by 2 equal doses per day at 9:00 and 17:00 from days 0 to 3. To evaluate AUC_{0-12h}, only 1 dose of MMF was administered at 9:00 on day 4. Animal weight was recorded each day before drug administration and weight growth during the 4-day MMF therapy was calculated as Weight Growth = (Weight_{d4} – Weight_{d0})/Weight_{d0} × 100% and then compared between treated groups and control groups. During therapy period, manifestations of anorexia (motivation in feed competition), vomiting, and diarrhea were recorded.

Blood Sampling

The blood samples were drawn from the jugular vein under general anesthesia. Anesthesia was induced by 5% isoflurane (Laboratoires Belamont, Paris, France) in O₂, and then maintained with 1% to 2% isoflurane in O₂. For measurement of MPA predose concentration (C₀), as well as examination of blood cell count and renal/hepatic functions, blood was collected on days 0, 2, 3, and 4 at 9:00 before MMF administration (thus MPA-C₀ was detected at 16 hours after the last dose of MMF).

On day 2, blood samples were also collected at 1, 2, 3, 4, and 6 hours after MMF administration to measure MPA-AUC_{0-6h}. On day 4, for evaluation of MPA-AUC_{0-12h}, blood samples were performed at 1, 2, 3, 4, 6, 8, 10, and 12 hours after drug administration.

Complete Blood Count and Examination of Renal/Hepatic Functions

On days 0, 2, 3, and 4, a complete blood count was performed using a Sysmex XT-2000iV™ veterinary hematology analyzer (Sysmex Europe GmbH, Norderstedt, Germany) following a specific veterinary protocol in Laboratory Biomédicale Biovelys (VetAgro Sup-Campus Vétérinaire de Lyon), whereas hepatic and renal functions were evaluated by measuring serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), and blood urea nitrogen (BUN) using a KONELAB KL20 ISEND automatic analyzer (Thermo Clinical Labsystems, Vantaa, Finland) in the same laboratory.

Plasma MPA Concentrations

For MPA plasma concentration measurement, blood samples were collected and stored at 4°C until centrifugation (<60 minutes after collection). After centrifugation, the plasma samples were stored at -80°C until MPA assay.

MPA plasma concentrations were determined using high-performance liquid chromatography (HPLC) equipped with diode array ultraviolet and mass spectrometer detectors. Briefly, after protein precipitation by acetonitrile and centrifugation, samples were evaporated under nitrogen stream. Dry residues were dissolved in the mobile phase and injected. Calibration curves were established with commercial calibration standards (0 to 5.28 mg/L). MPA concentrations were calculated from the signals obtained with the two detectors to ensure the purity of chromatographic peaks. Accuracy and precision were evaluated at three levels of quality control concentrations (0.463 - 2.35 - 4.72 mg/L). The inter-day precision was less than 6% with a bias less than 3%.

Measurement of MPA Variability

The measurement of variability of MPA concentration and MPA-AUC was performed according to previous study [5]: firstly, the interquartile range (IQR: the difference between the upper quartile and the lower quartile) was determined; subsequently, a nonparametric standard deviation was calculated by the Tukey method: $\sigma = \text{IQR}/1.35$, and then a nonparametric coefficient of variation (CV_{np}) was calculated: $\text{CV}_{np} = \sigma/\text{median} (\%)$.

Statistical Analysis

Data including animal weight growth, hepatic/renal function parameters, and blood cell counts are expressed as mean \pm SD. For these results, the differences between means of two data sets were analyzed by the Student *t* test, whereas multiple comparisons were subjected to a one-way analysis of variance and a least-significant difference test. A *P* value of < .05 was considered statistically significant.

MPA concentration and AUC were presented mainly by mean \pm SD, a description of median and range was also introduced. Correlations between variables were assessed by Spearman's rank coefficient (ρ). Variables were considered to be highly correlated when Spearman's ρ was > 0.8. Regression of MPA concentrations (C_0 or C_{max}) and MPA-AUC_{0-12h} was performed in a linear model.

All statistical analyses were performed using SPSS Statistics Version 17.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

MMF Has no Severe, Acute Effects in Newborn Pigs

MMF did not cause any obvious acute manifestation in all treated groups. MMF-treated piglets did not show noticeable anorexia. No vomiting or diarrhea was observed and no piglets died during therapy.

Compared to the control group, a dose-dependent decrease in weight growth can be observed in treated groups (Fig 1). However, this difference did not reach statistical significance (*P* < .05).

Concerning parameters of hepatic and renal functions (Fig 1), we did not find that any dose-related, significant change of AST, ALT, Cr, and BUN was observed in all treated groups.

MMF Reduces Lymphocyte and Monocyte Counts in Newborn Pigs

In neonatal pigs, short-term administration of MMF may cause alteration in several peripheral blood cells. Two types of comparisons were performed to confirm the alteration caused by MMF: comparison of blood cell count on day 4 versus day 0 within the same group, as well as comparison of blood cell count on day 4 between each treated group versus control group (Fig 2).

MMF-treated groups showed a dose-related decrease of lymphocyte count after 4 days of treatment with a statistically significant difference (*P* < .05) in group 3 (2 g/m²/d). In addition, we observed a significant decrease of monocyte count caused by MMF administration in groups 2 and 3 (1 g/m²/d and 2 g/m²/d, respectively) but not in group 1 (0.5 g/m²/d). MMF treatment did not cause a dose-dependent alteration in neutrophil count and total leukocyte count.

A significant reduced red blood cell count and hemoglobin concentration were observed in group 2 (1 g/m²/d) during MMF treatment. However, such effect was not observed in group 3 (2 g/m²/d). No significant change was observed in platelet count.

MPA Trough Concentrations

During days 2, 3, and 4, the MPA- C_0 of groups 1, 2, and 3 were 0.96 ± 0.32 , 2.27 ± 1.22 , and 5.47 ± 3.07 $\mu\text{g/mL}$, respectively (Table 1, Fig 3). Values of CV_{np} of MPA- C_0 were 37.40% (group 1), 92.30% (group 2), and 85.47% (group 3), indicating a high variability of MPA- C_0 , especially in groups 2 and 3. MPA- C_0 was not highly correlated to MMF dose ($\rho = 0.786$); moreover, because of high MPA- C_0 variability, the estimate of MPA- C_0 by MMF dose was not reliable using the linear regression model ($r^2 = 0.521$).

MPA Maximum Concentrations

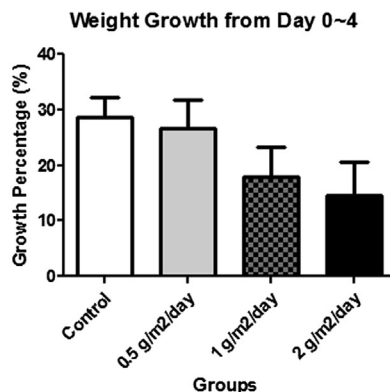
MPA maximum concentrations (MPA- C_{max}) calculated with data from both days 2 and 4 were 5.58 ± 1.39 , 11.54 ± 4.56 , and 19.48 ± 9.36 $\mu\text{g/mL}$, in groups 1, 2, and 3, respectively. Regarding MPA- C_{max} data, 11 of 18 of them were achieved at 1 hour after drug administration, 5 of 18 at 2 hours, and 2 of 18 at other time points (Table 1, Fig 3). Thus, the time to MPA- C_{max} (time-to-peak) can be considered to be 1 to 2 hours, similar to the results observed in human patients of whom the MPA- C_{max} usually occurred within 0.5 to 2 hours after drug intake [10]. Values of CV_{np} of MPA- C_{max} were 24.76% in group 1, 51.08% in group 2, and 38.39% in group 3, less than the CV_{np} of MPA- C_0 during days 2, 3, and 4. Similar to MPA- C_0 , a high correlation between MPA- C_{max} and MMF dose was not observed ($\rho = 0.656$) and estimation of MPA- C_{max} by MMF dose was not reliable using the linear regression model ($r^2 = 0.508$).

Concentration-Time Curves and MPA-AUC

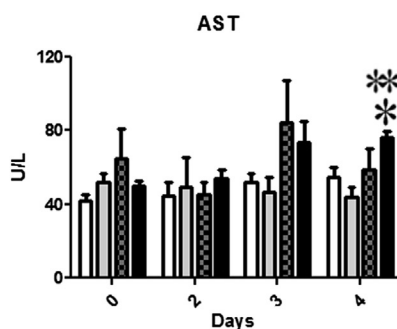
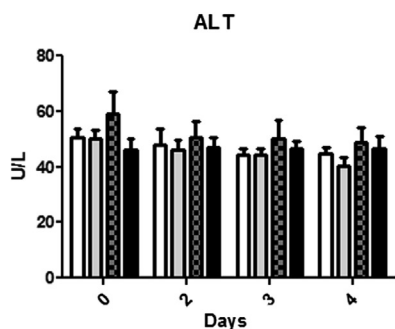
In groups 1, 2, and 3, MPA-AUC_{0-6h} (analyzed with data from both days 2 and 4) were 17.85 ± 4.60 , 39.69 ± 17.65 ,

A Weight Growth

- Group 0: Control Group
- ▒ Group 1: 0.5 g/m²/day
- ▓ Group 2: 1 g/m²/day
- Group 3: 2 g/m²/day



B Hepatic Function



C Renal Function

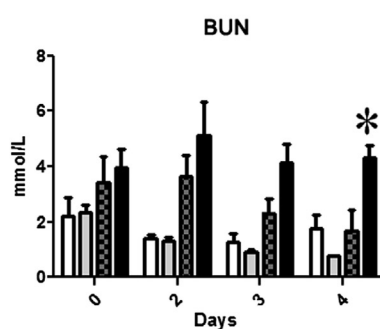
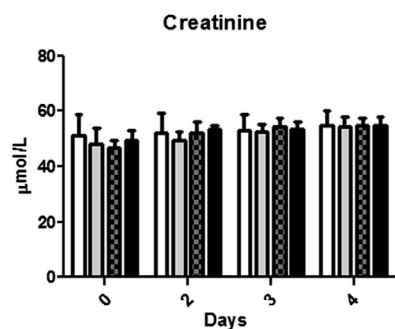


Fig 1. MMF has no severe, acute effect in neonatal pigs. Single asterisk signifies a significant difference ($P < .05$) in comparison of blood cell count on day 4 versus day 0 within the same group, while double asterisks refer to a significant difference ($P < .05$) in comparison of blood cell count on day 4 between each treated group versus control group. **(A)** Weight growth. Animals in control group grew $28.7 \pm 6.1\%$ from day 0 to day 4, while animals grew $26.7 \pm 8.8\%$ in Group 1, $17.9 \pm 9.3\%$ in Group 2 and $14.7 \pm 10.2\%$ in Group 3. **(B)** Hepatic function. In Group $2\text{g/m}^2/\text{day}$, AST on d4 showed a significant increase compared to d0; but it was also significantly higher than AST of control group on d4. **(C)** Renal function. In Group $2\text{g/m}^2/\text{day}$ the BUN on d4 was also significantly higher than all other groups; however, it didn't show an increase compared to d0.

and $74.53 \pm 29.83 \mu\text{g}^*\text{h/mL}$, respectively. Measurements of MPA-AUC_{0-12h} on day 4 were 22.00 ± 3.32 , 57.57 ± 34.30 , and $140.00 \pm 19.70 \mu\text{g}^*\text{h/mL}$, respectively (Table 1, Fig 3).

Values of CV_{np} of MPA-AUC_{0-6h} were 20.01% in group 1, 45.58% in group 2, and 20.99% in group 3. MPA-AUC_{0-6h} and MMF dose was not highly correlated ($\rho = 0.774$). Estimation of MPA-AUC_{0-6h} by MMF dose was not reliable using the linear regression model ($r^2 = 0.614$). MPA-AUC_{0-6h} was highly correlated with MPA-C₀ ($\rho = 0.932$, $P < .01$) and MPA-C_{max} ($\rho = 0.888$, $P < .01$).

Similar to MPA-AUC_{0-6h}, MPA-AUC_{0-12h} at day 4 was also highly correlated with MPA-C₀ ($\rho = 0.933$, $P < .01$) and MPA-C_{max} ($\rho = 0.900$, $P < .01$). Thus, the linear regression of MPA-C₀ or MPA-C_{max} to MPA-AUC_{0-12h} was performed.

Results showed that $\text{AUC}_{0-12h} = 17.882 \times C_0 + 14.479$, $r^2 = 0.966$, and $\text{AUC}_{0-12h} = 8.689 \times C_{\text{max}} - 29.141$, $r^2 = 0.940$.

DISCUSSION

This study described the pharmacokinetics profiles and the immunosuppressive effect on homeostasis-driven lymphocyte proliferation, as well as the side-effects of MMF observed in the neonatal swine model. This experiment was performed to define a proper, safe dose of MMF and an applicable monitoring strategy for immunosuppressive therapy in experimental transplantation studies with newborn pigs.

MMF is widely used in maintenance immunosuppressive therapy in pediatric solid organ transplantation. Generally,

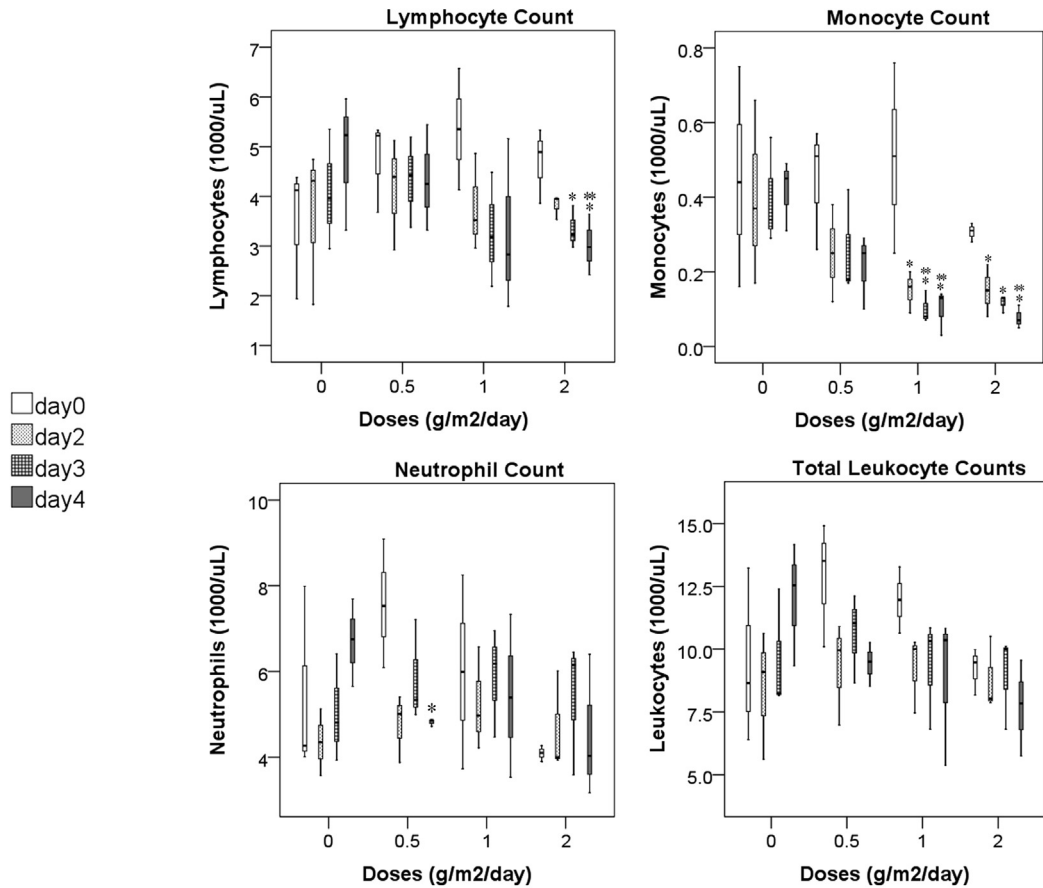
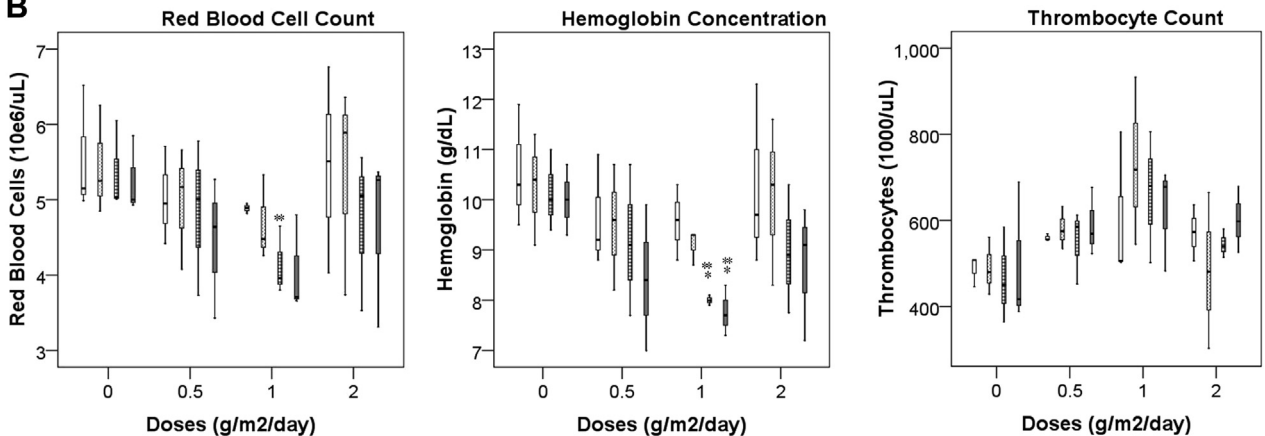
A**B**

Fig 2. Alteration of blood cell counts by short-term treatment of MMF in neonatal pigs. Unit was 1000/ μ L except special mark. Single asterisk signifies a significant difference ($P < .05$) in comparison of blood cell count on day 4 versus day 0 within the same group, while double asterisks refer to a significant difference ($P < .05$) in comparison of blood cell count on day 4 between each treated group versus control group. **(A)** Leukocyte sub-populations and total leukocyte count. (a) Lymphocytes: in contrast to control group in which lymphocyte count increased spontaneously from $(3.48 \pm 1.34) \times 10^3/\mu\text{L}$ to $(4.84 \pm 1.36) \times 10^3/\mu\text{L}$, Group 0.5 g/m²/day showed a stable lymphocyte count. In Group 2 g/m²/day, statistically significant decrease ($P < .05$) of lymphocyte count was observed, from $(4.69 \pm 0.75) \times 10^3/\mu\text{L}$ to $(3.34 \pm 0.43) \times 10^3/\mu\text{L}$. Steady decrease of lymphocyte count was observed in Group 1 g/m²/day from $(5.35 \pm 1.22) \times 10^3/\mu\text{L}$ to $(3.26 \pm 1.73) \times 10^3/\mu\text{L}$, but statistical significance was not obtained. (b) Monocytes: significant decrease of monocyte count caused by MMF administration was observed in both Groups 1g/m²/day and 2g/m²/day. (c) Neutrophils: Although neutrophil count of day 4 in Group 0.5g/m²/day showed a decrease compared to day 0, it did not showed significant difference to control group. Moreover, no dose-dependent effect was observed by results from two other treated groups. **(B)** Red blood cell (RBC), hemoglobin and thrombocyte count. Compared to control group, Group 1g/m²/day showed significantly lower RBC count on day 3 and lower HGB concentration on day 3 and 4; however, such effect was not observed in Group 2g/m²/day. No significant change was observed in platelet count.

Table 1. MMF Doses and MPA Exposure

	Group 1 (0.5 g/m ² /d)	Group 2 (1 g/m ² /d)	Group 3 (2 g/m ² /d)
C₀ (n = 27, µg/mL)			
Mean ± SD	0.96 ± 0.32	2.27 ± 1.22	5.47 ± 3.07
Median (minimum, maximum)	1.01 (0.47, 1.46)	1.89 (0.77, 4.04)	5.20 (1.44, 9.83)
CVnp	37.40%	92.30%	85.47%
C_{max} (n = 18, µg/mL)			
Mean ± SD	5.58 ± 1.39	11.54 ± 4.56	19.48 ± 9.36
Median (minimum, maximum)	5.325 (4.34, 8.19)	11.76 (5.60, 17.25)	19.67 (4.13, 33.42)
CVnp	24.76%	51.08%	38.39%
AUC_{0-6h} (n = 18, µg*h/mL)			
Mean ± SD	17.85 ± 4.60	39.69 ± 17.65	74.53 ± 29.83
Median (minimum, maximum)	16.29 (13.62, 26.15)	36.54 (19.65, 60.68)	80.21 (19.05, 105.78)
CVnp	20.01%	45.58%	20.99%
Correlation with C ₀ or C _{max}	with C ₀ : ρ = 0.932 (P < .001); with C _{max} : ρ = 0.888 (P < .001)		
AUC_{0-12h} (n = 9, µg*h/mL)			
Mean ± SD	22.00 ± 3.32	57.57 ± 34.30	140.00 ± 19.70
Correlation, linear regression with C ₀	ρ = 0.933 (P < .001), AUC _{0-12h} = 17.882 × C ₀ + 14.479, r ² = 0.966		
Correlation, linear regression with C _{max}	ρ = 0.900 (P < .001), AUC _{0-12h} = 8.689 × C _{max} - 29.141, r ² = 0.940		

Abbreviations: C₀, trough concentration; C_{max}, maximum concentration; AUC, area under the concentration-time curve; SD, standard deviation; CVnp, nonparametric coefficient of variation.

the overall efficacy and tolerability of MMF in pediatric patients are comparable to those described in adult patients, except for a higher prevalence of gastrointestinal adverse effects in children younger than 6 years [3]. The currently recommended dose in pediatric patients with concomitant CsA is 1200 mg/m²/d in 2 divided doses; the recommended MMF dose with concomitant FK506 or without a concurrent CNI is 900 mg/m²/d in 2 divided doses [3,18]. As in adults, there is an approximately 10-fold variability in dose-normalized MPA-AUC₀₋₁₂ values in pediatric patients after renal transplantation, strengthening the argument for concentration-controlled dosing of the drug [10].

The currently recommended therapeutic window for MPA exposure in conjunction with full-dose CNI therapy in the initial period after pediatric renal transplantation to minimize the risk of acute rejection is 30~60 mg*h/L via HPLC or 37~70 mg*h/L via enzyme multiplied immunoassay technique (EMIT). Predose plasma concentrations (C₀) should be between 1.0 and 3.5 mg/L (HPLC) or 1.3 and 4.5 mg/L (EMIT) [19,20]. Maximum plasma concentrations (C_{max}) determined by an HPLC method should be at 14.5 ± 4.21 mg/L [21].

MMF is also administered in experimental transplantation studies conducted with a swine model [11-15]; however, the pharmacokinetics of MMF in pigs has never been reported. Moreover, concerning the therapeutic effects, MPA is primarily metabolized by glucuronidation via uridine diphosphate-glucuronosyltransferases (UGTs) in the gastrointestinal tract, liver, and kidney. Whether the same UGTs are expressed in swine species and have similar metabolic characteristics as in humans remains unknown.

Related to previous studies conducted in our laboratory focusing on transplant tolerance induction in neonates and infants [16], for this study we have chosen neonatal pigs as a preclinical model. Similar to mouse and human neonates, pig neonates are born with a low peripheral blood

lymphocyte count which is termed neonatal lymphopenia [22]. This neonatal lymphopenia subsequently results in homeostasis-driven proliferation of lymphocytes in peripheral blood [23]. Thus, a natural increase of blood lymphocyte count can be observed in newborn pigs. Theoretically, administration of MMF in neonatal pigs will interfere with the homeostasis-driven proliferation of lymphocytes, thus resulting in stable or even a decrease in the blood lymphocyte count. Therefore, the blood lymphocyte count can be used as parameter for evaluation of MMF immunosuppressive effects in newborn pigs.

In this study, 4 days of MMF therapy did not cause severe, acute hepatic or renal toxic effects. Although manifestations of vomiting and diarrhea were not observed, MMF-treated piglets showed dose-related, lower body weight growth, indicating a potential gastrointestinal side-effect of MMF.

Short-term administration of MMF effectively interfered with the lymphopenia-driven proliferation of lymphocytes in neonatal pigs. The lymphocyte count in the control group showed an increase from 3.48 ± 1.34 × 10³/µL to 4.84 ± 1.36 × 10³/µL (although not statistically significant). Corresponding to the increase of MMF dose, group 1 showed a stable lymphocyte count and group 3 a significant reduced lymphocyte count. Although statistical significance was not observed, a lymphocyte count decrease from 5.35 ± 1.22 × 10³/µL to 3.26 ± 1.73 × 10³/µL was observed in group 2. We thus considered that in neonatal pigs the immunosuppressive effect of MMF is efficient and dose-related. Besides, considering short-term MMF therapy with doses from 0.5 to 2 g/m²/d in this study, although a dose-related neutropenia was not evident, a significant reduction of monocyte count was observed in groups 2 and 3 but not in group 1.

The present study showed that MMF pharmacokinetics profiles in neonatal pigs have similar characteristics with those described in human patients. At days 2 and 4, 16 of 18 of the C_{max} were reached at 1 or 2 hours after MMF administration,

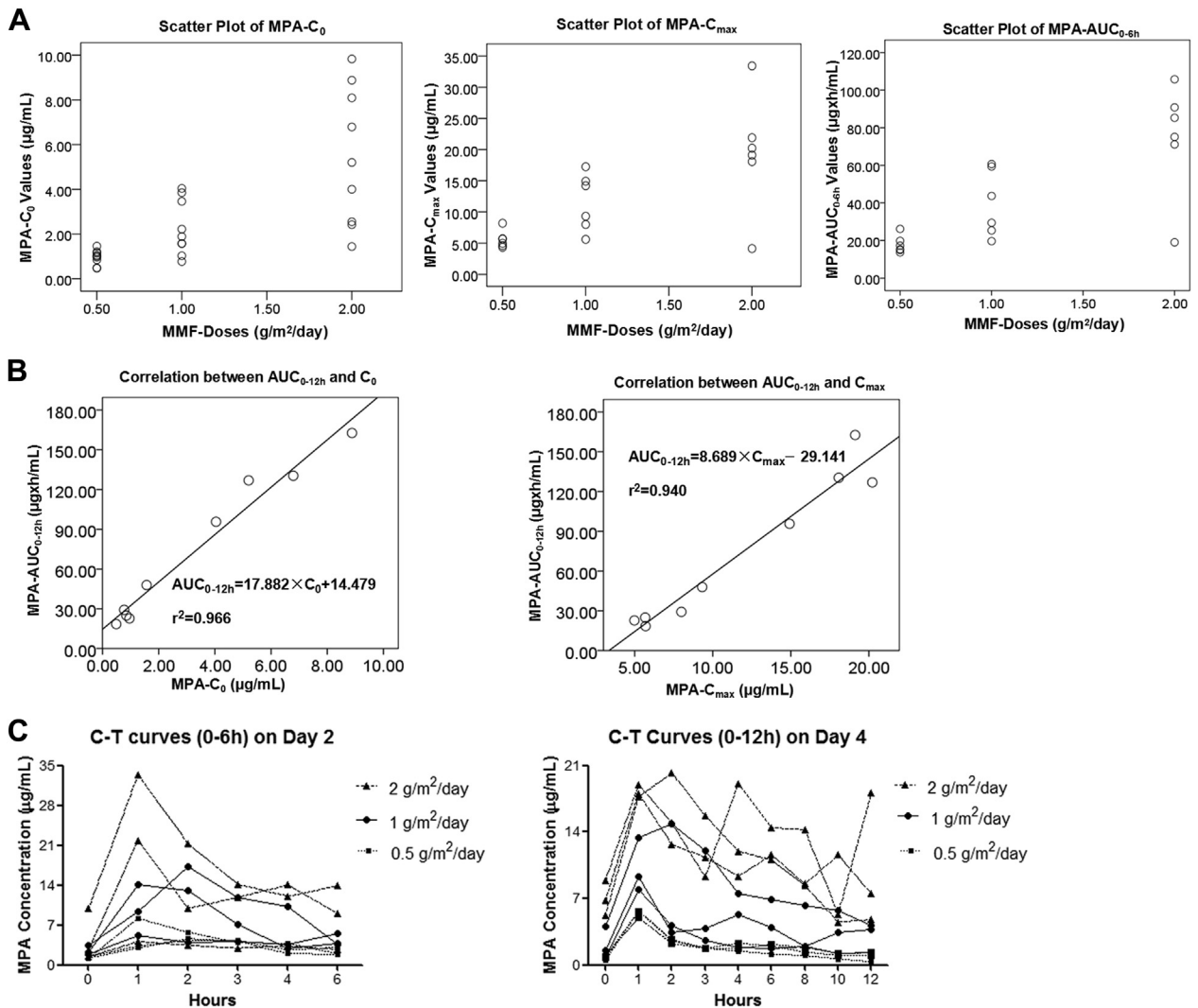


Fig 3. Pharmacokinetic profiles of MMF in neonatal swines. **(A)** Variability in dose-concentration relationship: MPA- C_0 was significant but not highly correlated to MMF dose (Spearman's $\rho = 0.786$, $P < .001$), indicating that increase of MMF dose will correspondingly result in elevated MPA- C_0 ; however, because of high MPA- C_0 variability, estimate of MPA- C_0 by MMF dose was not reliable by linear regression model ($r^2 = 0.521$). Similar dose-concentration relationship was also observed in MPA- C_0 (Spearman's $\rho = 0.656$, $r^2 = 0.508$ in linear regression model) and MPA- AUC_{0-6h} (Spearman's $\rho = 0.774$, $r^2 = 0.614$ in linear regression model). **(B)** Correlations between MPA- AUC_{0-12h} and MPA- C_0 or MPA- C_{max} : MPA- AUC_{0-12h} was highly correlated with MPA- C_0 ($\rho = 0.933$, $P < .01$) and with MPA- C_{max} ($\rho = 0.900$, $P < .001$). The linear regression of MPA- C_0 or MPA- C_{max} to MPA- AUC_{0-12h} was also performed. **(C)** C-T curves of plasma MPA concentration: MPA C-T curves showed high bioavailability of MMF in neonatal swines. The time to MPA- C_{max} (time-to-peak) was ≈ 1 hr, similar to the results observed in human patients.

indicating a high bioavailability of MMF in newborn pigs. High interindividual variability of each MPA pharmacokinetic parameter (C_0 , C_{max} and AUC_{0-6h}) was also confirmed. As in human patients, reasons for MPA pharmacokinetic variability may include differences in albumin, bilirubin, hemoglobin concentrations, renal and hepatic functions, as well as genetic polymorphisms in uridine diphosphate (UDP) – glucuronosyltransferase (UGT) expression. Co-administration of CsA inhibits transport of the metabolite mycophenolate glucuronide into the bile, results in less or no enterohepatic

recycling of MPA and hence leads to lower drug exposure; whereas FK506 does not interact with MPA.

MMF administration in piglets with the dose of $1 \text{ g/m}^2/\text{d}$ lead to a drug exposure corresponding to the approved MPA level in clinical transplantation setting, characterized by MPA- AUC_{0-12} of $30 \sim 60 \mu\text{g} \times \text{h/mL}$, MPA- C_0 of 1.5 to $3.5 \mu\text{g/mL}$ and MPA- C_{max} of 10 to $20 \mu\text{g/mL}$ (all detected by HPLC as in the present study). Besides, the MMF dose of $1 \text{ g/m}^2/\text{d}$ also maintains better balance between desired therapeutic effects and adverse side effects. We thus

concluded that an MMF dose at 1 g/m²/d may be used as an initial dose in immunosuppressive therapy.

Similar to pediatric patients, AUC_{0-6h}, C₀, and C_{max} of MPA have no high correlation with MMF doses in piglets. To achieve optimal immunosuppressive outcome, MPA exposure should be monitored and doses should be adjusted [10,18].

AUC_{0-12h} can be considered the most informative parameter of drug exposure; however, the blood sampling schedules for this monitoring strategy are not practicable in transplantation studies. Especially in experimental research with swine model, frequent blood collections may result in porcine stress syndrome, manifested by perturbation of the immune system which involves a sudden, severe increase or decrease of lymphocyte or neutrophil counts [24,25]. Therefore, MMF monitoring strategies in pigs should be balanced to their own harmful effect. From this aspect, MMF monitoring by one single time point is undoubtedly a more practical strategy than limited sampling strategies which required several blood samplings.

Both C₀ and C_{max} (≈ C₁) were shown to be significantly correlated to AUC_{0-12h}, thus are justified to be used for monitoring of MPA exposure. In transplantation studies, MMF is often administered with CsA or FK506 which are routinely monitored with C₀. By this consideration, C₀ monitoring that does not require once more blood sampling and anesthesia will cause less harmful intervention than C₁ monitoring; thus, it may be a more practical strategy for MMF surveillance in pigs. From the standpoint of a clinical pharmacokinetic study, MPA-C₀ is not a very informative parameter and not the best time point to choose for MPA monitoring; however, in an experimental study, C₀ monitoring may be the most practicable one.

In this study, to make the experiment schedule more convenient, MPA-C₀ was measured at 16 hours after the last dose of MMF (but not at 12 hours as a standard interval in clinical study). Importantly, on day 4 the MPA-C₀ at the 12-hour interval can be counted with 0.89 ± 0.48, 3.08 ± 1.50, and 10.14 ± 7.06 µg/mL in the three treated groups, and it was relatively higher than the MPA-C₀ measured at the 16-hour interval.

The other limitation is that in this study MMF was administered without concomitant therapies of CNIs and corticosteroids. The recommended MPA-AUC_{0-12h} of 30 to 60 µg·h/mL was derived from the clinical trial in adult renal transplantation patients receiving CsA, MMF, and corticosteroids [1]. In human patients, co-administration of CsA interferes with the MPA pharmacodynamics and leads to lower MPA exposure, as mentioned above. Thus, the recommended initial dose of 1 g/m²/d has to be confirmed in future transplantation studies if co-administered with CsA. Besides, a longer time of MMF therapy is required to study the evolution of MMF pharmacokinetics, immunosuppressive effects, and side effects to adjust the MMF dosage in long-term study.

CONCLUSIONS

As in pediatric patients, oral MMF has high bioavailability in neonatal swine; however, high interindividual variability

in the dose-concentration relationship was also confirmed. MMF administered at proper doses can cause a decrease in lymphocyte and monocyte counts in neonate pigs; in the meantime, less body weight growth was also observed. By balancing the therapeutic and side effects, an MMF dose of 1 g/m²/d is recommended as an initial dose for immunosuppressive therapy in piglets, which leads to the MPA-AUC_{0-12h} of 30~60 µg × h/mL. With the current doses of MMF used in this study, a predose MPA concentration (C₀) was proven to be significantly correlated to MPA-AUC_{0-12h}; thus C₀ monitoring can serve as a practical strategy for surveillance of MPA exposure.

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