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/m^2/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/m^2/d reduced both lymphocyte and monocyte counts of piglets. Oral MMF had high bioavailability in neonatal swine. MPA-$AUC_{0\text{-}12h}$ of doses 0.5, 1, and 2 g/m^2/d was 22.00 ± 3.32, 57.57 ± 34.30, and 140.00 ± 19.70 μg × h/mL, respectively. Neither MPA trough concentration (MPA-C_0), nor MPA maximum concentration (MPA-C_{max}) or MPA-$AUC_{0\text{-}6h}$ had high correlation with MMF-dose. For surveillance of MPA exposure, MPA-C_0 had significant correlation with MPA-$AUC_{0\text{-}12h}$ (Spearman's $\rho = 0.933$, $AUC_{0\text{-}12h} = 17.882 \times C_0 + 14.479$, $r^2 = 0.966$).

Conclusion. To reach adequate drug exposure and to reduce dose-dependent side effects, an MMF dose of 1 g/m^2/d is recommended to be used as an initial dose for immunosuppressive therapy in piglets, and MPA-C_0 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-$AUC_{0\text{-}12}$) 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 intrapatient 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/m^2/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 $AUC_{0\text{-}12h}$, 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 = $(Weight_{d4} - Weight_{d0})/Weight_{d0} \times 100\%$ 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 O_2, and then maintained with 1% to 2% isoflurane in O_2. For measurement of MPA predose concentration (C_0), 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-C_0 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-$AUC_{0\text{-}6h}$. On day 4, for evaluation of MPA-$AUC_{0\text{-}12h}$, 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.