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Monitoring of mycophenolate mofetil metabolites in children with nephrotic syndrome and the proposed novel target values of pharmacokinetic parameters

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ABSTRACT

The aim of the study was to estimate target values of mycophenolate mofetil (MMF) pharmacokinetic parameters in children with proteinuric glomerulopathies by calculating the pharmacokinetic parameters of MMF metabolites (mycophenolic acid [MPA], free MPA [fMPA] and MPA glucuronide [MPAG]) and assessing their relation to proteinuria recurrence.

One hundred and sixty-eight blood samples were collected from children, aged 3–18 years, diagnosed with nephrotic syndrome or lupus nephritis. MMF metabolites concentrations were examined before drug administration (C_{trough}) and up to 12 h afterward employing high-performance liquid chromatography.

Dose-normalized MPA C_{trough} and area under the concentration–time curve from 0 to 12 h (AUC_{12}) were within 0.29–6.47 μg/mL/600 mg/m^2 and 9.97–105.52 μg h/mL/600 mg/m^2, respectively. MPA C_{trough} was twofold lower ($p = 0.024$) in children with proteinuria recurrence. MPA, fMPA and MPAG concentrations correlated positively to respective AUC_{12}.

It may be suggested MMF metabolites monitoring in children with proteinuric glomerulopathies is justified by MPA $C_{trough} < 2$ μg/mL in patients at risk of the proteinuria recurrence. Such a recurrence is most probably caused by not sufficient MPA concentration during proteinuric glomerulopathies treatment. MPA $C_{trough} > 3$ μg/mL may be considered as an efficient one to avoid proteinuria recurrence. Finally, MPA target AUC_{12} should exceed 60 μg h/mL to ensure the safe and effective treatment in children with nephrotic syndrome, however, the upper limit is still to be established.

1. Introduction

An immunosuppressant, mycophenolate mofetil (MMF), is used after renal, liver or heart transplantation (Tönshoff et al., 2005; Staatz and Tett, 2007; Höcker et al., 2005; Aw et al., 2008) to prevent the acute organ rejection both in adult and pediatric patients (Zimmerhackl et al., 2006). MMF is one of the standard immunosuppressive drugs due to its great treatment efficacy (Irving and Webber, 2010; Jungraithmayr et al., 2007) and limited toxicity e.g. on liver or kidney. The main MMF adverse effects include gastrointestinal and hematological disorders (Tönshoff et al., 2005). MMF usage enables the reduction or the withdrawal of steroids reducing their toxicity (Tönshoff et al., 2005). Therefore, MMF has been recently more commonly used in autoimmune diseases, childhood nephrotic syndromes with different etiologies, lupus nephritis as well as vasculitis therapies (Gargah and Lakhoua, 2011; Afzal et al., 2007; Filler et al., 2003; Ostalska-Nowicka et al., 2011; Li et al., 2010; Nickavar et al., 2012; de Mello et al., 2010; Kazyra et al., 2010). MMF is also suitable for long-term treatment as it does not cause nephrotoxicity, which is frequently observed after cyclosporine (CsA) (Fujinaga et al., 2007). Moreover, MMF may substitute CsA and cyclophosphamide in some children with lupus nephropathy (Ostalska-Nowicka et al., 2011).

Pharmacokinetics of MMF main metabolite, mycophenolic acid (MPA), is complex. Firstly, although MPA glucuronide (MPAG) is pharmacologically inactive, it undergoes the enterohepatic

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recirculation. It results in the secondary MPA concentration peak 6–12 h after MMF administration, which may account for about 10–60% of the total MPA AUC. Secondly, MPA is highly protein bound (97–99%) and only unbound, free fraction (fMPA) is pharmacologically active (Chen et al., 2010; Downing et al., 2013; Tönshoff et al., 2011). Thirdly, pharmacokinetic parameters of MPA, MPAG and acyl glucuronide of MPA, show high (approximately 10-fold) inter- and intrapatient variability in both adults and children (Parant et al., 2009; Tönshoff et al., 2011; Weber et al., 2008, 2002a,b). Moreover, there are significant differences in the MPA pharmacokinetics between adult and pediatric patients (Parant et al., 2009; Tönshoff et al., 2011; Weber et al., 1998). The variation in the expression of the UDP-glucuronosyltransferases during development is probably related to the changes in MPA clearance. The differences are apparent especially in children younger than 10 years of age and adults (Parant et al., 2009; Filler et al., 2008). As a result, younger patients require higher MPA doses per body surface area (Filler, 2007) as well as more frequent dosing than adolescents and adults, which probably results from age-dependent variations in the transporter, P-glycoprotein, implicated in reduced absorption of medications (Downing et al., 2013). It remains unclear whether the target exposure is the same in children and adults (Filler, 2007), especially as the target MPA AUC values in patients with autoimmune diseases has not been established yet (Abd Rahman et al., 2014). Additionally, the variability of MMF metabolites pharmacokinetics in children may be affected by various factors (treatment duration, therapeutic indication, drugs co-administered, genetic, physiological and environmental factors as well as kidney or liver dysfunction) (Filler, 2007, 2006; Parant et al., 2009; Brown et al., 2002; Jacobson et al., 2008; Ghio et al., 2009). There is little data in the literature on the pharmacokinetics of MMF metabolites in children (Filler, 2007, 2006), especially diagnosed with nephrotic syndrome (Zhao et al., 2010; Saint-Marcoux et al., 2011). Additionally, food intake may be one of the factors influencing MMF pharmacokinetics. According to the manufacturer information, food had no effect on the extent of MPA absorption in renal transplant recipients. However, MPA C_{max} decreased by 40% and, therefore, MMF should be administered on an empty stomach early after transplantation. In stable patients, MMF may be administered with food if necessary (Staatz and Tett, 2007). Circadian variations may also be one of the factors influencing MMF variability. In a study on rats, it was shown that MPA C_{max}, AUC from 0 to 24 h (AUC_{0-24}) and plasma clearance varied significantly according to the circadian dosing-time (Dridi et al., 2014).

The factors described above cause difficulties in obtaining the optimal MPA exposure. The insufficient exposure, which is often seen, leads to lack of efficacy. On the other hand, overexposure may be associated with unacceptable side effects of MMF (Parant et al., 2009; Weber et al., 2008, 2002a), such as gastrointestinal (nausea, upper abdominal pain, diarrhea) and hematologic disorders (leukopenia, anemia), and may lead to dose reduction or treatment discontinuation (Tönshoff et al., 2011). Therefore, in order to enhance the efficiency and safety of MMF therapy, therapeutic drug monitoring (TDM) is more frequently proposed (Filler, 2006). However, there is still ongoing debate whether TDM is justified in case of MMF (Staatz and Tett, 2014) and the therapeutic window for MPA AUC_{12} in autoimmune disease patients has not been established yet (Abd Rahman et al., 2014).

In this study, we aimed to estimate the target values of MPA pharmacokinetic parameters in children with proteinuric glomerulopathies by determining full 12 h pharmacokinetic profiles, calculating the pharmacokinetic parameters of MPA as well as fMPA and MPAG and assessing their relation with proteinuria recurrence. We also analyzed the relations between MPA, fMPA and MPAG pharmacokinetics and some clinical factors as well as biochemical parameters. Additionally, we decided to determine MPAG concentration to check the influence of MPAG on MPA AUC in studied children and to describe the pharmacokinetics of MMF metabolites comprehensively.

2. Methods

2.1. Study population

The study included 24 pediatric patients aged 3–18 years with idiopathic nephrotic syndrome ($n = 20$) and lupus nephritis ($n = 4$), treated with MMF and corticosteroids. Patients were hospitalized in Department of Pediatric Nephrology, University of Medical Sciences in Poznan, Poland. MMF was administered orally twice a day at the maximum dose of 1200 mg/m^2/day (2 g/day). The inclusion criteria were the appropriate MMF dosage (the same dose twice a day) and the duration of treatment with unchanged MMF dose for at least one month prior to the pharmacokinetic study. The exclusion criteria was different MMF dosage schedule than twice a day or at two different doses. The children's demographics and biochemical characteristics are presented in Table 1.

On the day of blood collection, proteinuria (less than 50 mg/kg body mass/day) was observed in four of the 24 children. In six children hematological disorders occurred, anemia, leukopenia, and leukocytosis in four, two and three children, respectively, which in two patients led to a reduction in the dose of MMF. We did not observe any gastrointestinal disorders. Anemia, leukopenia, and leukocytosis were defined as hemoglobin concentration <12 g/dl, decrease in white blood cells count $<4.0 \cdot 10^9$/l, and increase in white blood cells count $>10.0 \cdot 10^9$/l, respectively.

Blood samples were collected into EDTA tubes before MMF administration (C_{trough}) and subsequently 1 h (C_1), 2 h (C_2), 3 h (C_3), 4 h (C_4), 6 h (C_6), 9 h (C_9) and 12 h (C_{12}) after its administration. In 12 children blood samples were collected up to 6 h, therefore, it was assumed that C_{12} was equal to C_{trough} as MMF was administrated in 12-h intervals (David-Neto et al., 2003). In total, one hundred and sixty-eight blood samples were analyzed. The samples were centrifuged to obtain plasma, immediately frozen and kept at −20 °C until analysis. The study was approved by the Bioethical Committee at Poznan University of Medical Sciences and it is in accordance with the 1964 Declaration of Helsinki and

Table 1
Children's demographic and biochemical characteristics.

Parameter	Median	Range
24 children	Male (12)/female (12)	
Age (years)	10	3–18
Body weight (kg)	33	15–70
Treatment indication	Nephrotic syndrome, $n = 20$ Lupus nephritis, $n = 4$	
Proteinuria	Yes, $n = 4$ No, $n = 20$	
MMF dose (b.i.d.) (mg/m^2)	450	169–610
MMF dose (b.i.d.) (mg/kg)	15.5	4.8–23.1
Duration of MMF treatment (months)	4	1–32
Protein concentration (g/dL)	6.8	5.6–7.6
Glomerular filtration rate (mL/min/1.73 m^2)	129.7	98.6–183.4
Creatinine concentration (mg/dL)	0.43	0.23–0.75
Urea concentration (mg/dL)	22	9–42
Leukocytes count (10^{-9}/L)	7.21	3.46–17.28
Erythrocytes count (10^{-12}/L)	4.66	4.20–5.54
Platelet count (10^{-9}/L)	338	238–636
Hemoglobin (g/dL)	13.5	11.5–16.3
Hematocrit (%)	38.4	33.6–46.9
Alanine aminotransferase (U/L)	11	5–21
Aspartate aminotransferase (U/L)	26	18–34

MMF mycophenolate mofetil.