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Non-steroidal Anti-Inflammatory Drugs may reduce Enterohepatic Recirculation of Mycophenolic Acid in Patients with Childhood-onset Systemic Lupus Erythematosus

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Abstract

Background—The large inter-individual differences observed in mycophenolic acid (MPA) pharmacokinetics (MPA-PK) are in part attributed to large variability in enterohepatic recirculation (EHC) of the drug. MPA's main metabolite, MPA-glucuronide is actively secreted into the bile via the multidrug resistance-associated protein 2 (MRP2) transporter. Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit the MRP2 transporter which can alter EHC and drug exposure. Here, we evaluated the effects of this potential drug-transporter interaction on MPA-PK in a cohort for patients with childhood-onset systemic lupus erythematosus (cSLE) on mycophenolate mofetil (MMF) therapy.

Methods and Materials—Full MPA concentration-time profiles and demographics including co-medications were available for 19 patients with cSLE. Concentrations at pre-dose (C_{trough}), 9 hour (C_9) and nadir (C_{nadir} ; defined as the lowest concentration between C_{max} and C_9), and Area under the curve (AUC_{0-12} and AUC_{6-12}) were assessed using standard methods (WinNonlin5.1). AUC_{6-12}/AUC_{0-12} and C_9/C_{nadir} ratios were used to evaluate the effect of NSAIDs treatment on MPA-PK.

Results—Eleven out of 19 patients were on NSAID treatment, and did not show visual evidence of EHC in their PK profile. In contrast, patients not on NSAID therapy showed evidence of EHC-related MPA concentration increase in the later part of their PK profiles, typically after 6h. This phenomenon could be well characterized by the C_9/C_{nadir} ratio, which was significantly lower in the NSAID-treated cohort ($P < 0.01$).

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Conclusion—These preliminary data suggest that concomitant intake of NSAID may lower EHC of MPA possibly through inhibition of MRP2 transport of MPA-G. Further mechanism-based studies are needed to further elucidate this potential transporter interaction.

Keywords

Mycophenolic acid; pediatric patient; SLE; pharmacokinetics; Non-steroidal anti-inflammatory drug; multidrug resistance-associated protein 2

INTRODUCTION

Mycophenolate mofetil (MMF) is an immunosuppressive pro-drug commonly used in solid organ transplantation [1, 2] which is increasingly used off-label in the treatment of childhood-onset systemic lupus erythematosus (cSLE) [3]. After oral administration, MMF undergoes rapid conversion to its active form, mycophenolic acid (MPA) [1]. In kidney transplant recipients, MPA overexposure has been associated with adverse events (AEs) such as gastrointestinal complications (i.e. diarrhea) and leucopenia [2] while underexposure increases the risk of acute rejection. A strong relationship between MPA exposure, e.g. as measured by the area under the concentration curve (AUC), and drug efficacy as well as toxicity has been documented in transplant recipients and also in patients with cSLE.

The large unexplained inter-individual variability in MPA exposure emphasizes the special need to better understand factors contributing to this variability [1]. To date, genetic polymorphisms in uridine-glucuronosyl transferase metabolic enzymes (UGTs) [4], and transporters such as the multidrug resistance-associated protein 2 (MRP2, ABCC2) [5] have been identified to contribute to the PK variability. Another potential mechanism of increased variability is through drug-drug interaction of concomitant medications [6, 7]. For instance, cyclosporine combination therapy results in lower MPA exposure through inhibition of the transporter MRP2, thus reducing the contribution of enterohepatic recirculation (EHC) [6]. By contrast, an EHC-related secondary peak is often observed in patients on concomitant tacrolimus. EHC of MPA also appears to be affected by presence of a genetic polymorphism in the ABCC2 gene that the ABCC2(MRP2)-24C>T polymorphism is associated with significantly higher dose-corrected MPA trough levels [5]. This has been observed in patients on tacrolimus, but not on cyclosporine [4]. The most likely explanation for this observation is that the inhibition of MRP2 by cyclosporine masks the effect of the MRP2 genetic polymorphism.

Recently, it was reported that non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the MRP2- and MRP4-mediated methotrexate transport [8]. Actually, NSAIDs are frequently prescribed in patients with cSLE. As MRP2 has been identified as the major contributor to the EHC of MPA, we hypothesized that concomitant NSAIDs may influence the pharmacokinetic behavior of MPA in these patients. This study examines the effects of NSAIDs on the MPA-PK in relation to EHC in patients with cSLE.

MATERIALS AND METHODS

Patients

Pharmacokinetic and demographic data were obtained from a cohort of 19 patients with cSLE on MMF therapy as recently described [3]. The study was approved by the institutional review boards of the Cincinnati Children's Hospital Medical Center and Children's Memorial Hospital, Chicago, IL. Patients fulfilled the American College of Rheumatology Classification Criteria for SLE prior to the age of 16 years and were on an oral regimen of MMF as part of standard of care, with stable dosing and stable renal function for at least 3 weeks. Patients were permitted to continued use of other SLE medications, including NSAIDs. These medications were at a stable dosing for at least 30 days before study participation. Patients with medical conditions or prior surgery that could interfere with the pharmacokinetics of MMF as determined by the principal investigator were excluded. No patient had acute illness or infection within 5 days of study visits and took antibiotics. Relevant demographics and other factors such as adherence, concomitant medications and food intake were obtained from medical records, and drug and food diary. A drug diary was kept by the patients for three days prior the PK sampling day to indicate dosing history of MMF and concomitant medications.

Sampling for MPA-PK and Assay of Mycophenolic Acid and MPA-G Concentrations in Plasma

Patients were asked to record the time of MMF intake the preceding night, followed by a fast of at least 8 hours. MMF was taken with water or juice (orange or apple). Blood samples were obtained from an indwelling line (peripheral) or by direct venipuncture within 1 hour before the morning MMF dose (trough) and at 20 minutes, 40 minutes, and at 1, 1.5, 2, 3, 4, 6, and 9 hours after the administration. Patients had standard meals 1 hour (breakfast) and 4 hours (lunch) later on the PK sampling day.

The pharmacokinetics of MPA and the metabolite MPA-glucuronide (MPA-G) in plasma were analyzed using a validated high performance liquid chromatography (HPLC) assay performed at the Laboratory of Applied Pharmacokinetics and Therapeutic Drug Management (LAP-TDM) at CCHMC as previously described[3].

Pharmacokinetic Analysis

Standard non-compartmental analysis was used (WinNonlin Pro, version 5.1, Pharsight, Mountain View, California) to evaluate the pharmacokinetics of MPA and MPAG. Parameter included were: peak MPA concentration (C_{max}); trough MPA concentration (C_{trough}); MPA concentrations at 9 hour (C_9); nadir concentration between the peak and at 9 hour (C_{nadir}); the area under the curve (AUC), and CL/F (total body clearance divided by bioavailability). The AUC was calculated over 9 hours (AUC_{0-9}), for 12 hours (AUC_{0-12}) and for 6 hours from 6 to 12 (AUC_{6-12}) by using trough concentration as a value at 12 hour. To evaluate EHC, AUC_{6-12}/AUC_{0-12} , C_{trough}/C_{nadir} and C_9/C_{nadir} were assessed.

Genotyping for ABCC2 (MRP2) -24C>T polymorphism

Genomic DNA was extracted from blood using standard procedures. The ABCC2(formerly, MRP2)-24C>T genotype was determined by TaqMan assay (Applied Biosystems, Foster City, CA, USA), after crossly validated by direct sequencing assay[5].

Statistical Analysis

Numerical variables were summarized by mean with standard deviation (SD). Binary and categorical variables were summarized by frequency (%). Parametric and non-parametric analyses were undertaken by using JMP8, a SAS-based statistic software, when needed.

RESULTS

Eleven of 19 patients were on NSAIDs (nabumetone, celecoxib, diclofenac or acetylsalicylic acid) during the study. To evaluate the effect of NSAIDs on MPA PK, patients were divided into two groups according to NSAIDs intake. Patient characteristics with co-medications and descriptive pharmacokinetic profiles are summarized by group in Table 1. There was no significant difference between the two groups except for the use of ACE inhibitors (Table 1). Figure 1 depicts arithmetic mean ($\pm$ SD) MPA and MPAG concentration-time profiles by group. The dip at 1 hour in the NSAID+ group is the result of averaging individual data points with different T_{max}. Presenting the data as geometric means did not make a difference (data not shown). When explored graphically, most of the patients on NSAIDs showed steadily decreasing MPA concentrations from the time the peak occurred until the final 9-hour observation point. In contrast, almost all patients not on NSAIDs showed rising MPA concentrations from the time the nadir concentration was observed until the last observation point, implying the occurrence of EHC. This effect on the PK profile was best captured by the C_9/C_{nadir} ratio, which showed a significantly lower ratio in NSAID-treated patients compared to patients not on NSAIDs ($P < 0.01$, Mann-Whitney U-test)(Figure 2). These phenomena could not be captured well using the AUC_{6-12}/AUC_{0-12} ratio which has been used before as a marker of EHC to assess existence of EHC itself and/or evaluate the effect of EHC on MPA-PK[9]. There was no significant difference in pharmacokinetic parameter estimates for CL and AUC of MPAG between the two groups (Table 1). Grouping based on the intake of ACE inhibitor did not have a major impact on any of the pharmacokinetic parameters evaluated (data not shown). Five patients were carriers of the ABCC2 -24C>T polymorphism in our cohort: 4 heterozygous and 1 homozygous. The genetic contribution to C_9/C_{nadir} ratio and trough level was not observed probably because four of the all carriers, including one homozygote, were on NSAIDs.

DISCUSSIONS

To the best of our knowledge, this is the first observational study to describe the effect of NSAIDs use on MPA-PK in patients on MMF therapy. This study was prompted by the virtual lack of secondary peaks in the MPA-PK profiles of our cSLE patients[3] as compared to our experience with kidney transplant recipients[10]. It is well-documented that concomitant cyclosporine use reduces or negates EHC [6]. Patients with cSLE, however, typically are not prescribed cyclosporine. Based on systematic review of the EHC literature,

especially as it relates to MRP2, NSAIDs were suspected as a possible culprit because of similar inhibitory activity of MRP2 as cyclosporine [8]. In this study, no significant difference in AUC₀₋₁₂ between patients with and without NSAID use was found, contrary to what has been reported for cyclosporine [6]. Patients on NSAIDs showed low C₉/C_{nadir} ratios as compared to patients without NSAIDs co-administration. The latter group had higher ratios with large variability (mean $\pm$ SD; 1.2 $\pm$ 0.4 vs. 2.4 $\pm$ 1.6, respectively), may be contributed to individual differences in intrinsic MRP2 activity. From these observations, it is speculated that cyclosporine may act as a stronger inhibitor of MRP2 than NSAIDs.

As for pharmacogenetic effect, the C₉/C_{nadir} ratio or C_{trough} levels for carriers of the ABCC2 -24C>T polymorphism as shown previously [5] was not significantly different. Four of the five ABCC2 -24C>T SNP carriers, however, were in the NSAID-treated group which showed low C₉/C_{nadir} ratios. A possible explanation for our observation could be that NSAIDs reduce the complexity of MPA-PK profile in terms of secondary peaks and therefore mask any ABCC2(MRP2) related pharmacogenetic effect, similar to the situation in patients on MMF and cyclosporine combination therapy. Thus, our finding of this drug-drug interaction would be valuable at least to better understand MPA kinetics so as not to misunderstand the other contributing factors (e.g. pharmacogenetic). Concerning co-medication of ACE inhibitors, some of them have a carboxylic acid in their chemical structure so they and their glucuronized metabolite are also a potential substrate of SLCO1B1 and ABCC2. Although no significant contribution of ACE inhibitor could be detected in current study due to small sample size, this needs to be further elucidated in a larger adequately powered study.

In summary, our preliminary data suggest a drug-drug interaction of MPA with NSAIDs that could explain in part inter-individual differences, especially the absence of secondary peaks of MPA. It is postulated that this interaction occurs through the alteration of ABCC2(MRP2) transport activity of MPA(G). Larger studies will be needed to confirm this observation and will provide new insight into factors contribution to variability in the EHC of MPA.

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Figure 1.

Mean (+/-SEM) MPA (A) and MPAG (B) concentration-time profiles. The open squares show mean PK profile in NSAID-treated patients (n=11), while closed squares show for non NSAID-treated patients (n=8). X-axis is displayed as a log scale. Most of the patients in the NSAID-treated patients had secondary rise in MPA concentration around 9 hour post-dose after reached at the lowest concentration between C_{max} and C_9 while non-treated patient showed a relatively simple PK profile.

Figure 2.

Individual C_9/C_{nadir} values. C_9/C_{nadir} was calculated from concentration at 9 hour post-dose (C_9) and the lowest concentration between the peak and C_9 (C_{nadir}). Non-parametric comparison revealed significant difference ($P < 0.01$) in C_9/C_{nadir} between the two groups.

Table 1

Patient characteristics and PK summary based on two groups

	NSAID (-)	NSAID (+)	P-value
MMF	8	11	
NSAIDs	0	11	
Number of patients	8	11	
Demographics (Mean and SD, or number of patients)			
Weight (kg)	61.5	6.9	70.3 18.2 0.16
Height (cm)	160.9	5.3	156.8 7.7 0.19
Age (years)	16.5	2.0	17.5 5.1 0.55
Gender (Male:Female)	(1:7)	(0:10)	0.42
Race(African American: Caucasian)	(4:4)	(7:4)	0.63
Ethnicity(Hispanic:Non-His)	(1:7)	(3:8)	0.60
Co-medications (number of patients, percentage)			
Prednisone	8 (100%)	10 (90.9%)	-
Methylprednisolone IV pulse	1 (12.5%)	2 (18.2%)	-
Aspirin	0	8 (72.7%)	-
Another NSAIDs ¹⁾	0	7 (63.6%)	-
Hydroxy-chloroquine	7 (87.5%)	10 (90.9%)	-
ACE-I ²⁾	6 (62.5%)	1 (9.1%)	<0.01 ⁵⁾
Ca-blocker ³⁾	2 (25.0%)	1 (9.1%)	-
Anti-acids ⁴⁾	3 (37.5%)	4 (36.4%)	-
Descriptive PK parameter (Mean and SD)			
MPA			
C _{trough} (mg/L)	2.01	2.52	1.00 0.57 0.21
C ₃ (mg/L)	2.33	1.50	1.36 1.05 0.12
AUC ₀₋₁₂ (hr*mg/L)	34.8	24.7	30.0 12.4 0.58
AUC ₀₋₁₂ (hr*mg/L per MMF 1g dose)	35.9	24.5	31.4 11.9 0.60
CL/F (L/hr)	29.2	17.7	27.1 10.8 0.75

	NSAID (-)		NSAID (+)		P-value
MMF Dose (mg)	1000	267	1000	387	1.00
C ₀ /C ₁₂ mid	2.4	1.6	1.2	0.4	<0.01 ⁶⁾
AUC ₀₋₁₂ (hr*mg/L)	17.7	16.3	11.7	5.7	0.27
AUC ₀₋₁₂ /AUC ₀₋₁₂	0.47	0.19	0.39	0.11	0.28
MPAG					
CL/F (L/hr)	2.73	1.94	2.34	0.23	0.55
AUC ₀₋₁₂ (hr*mg/L)	357	228	323	135	0.69
AUC ₀₋₁₂ (hr*mg/L per MMF 1g dose)	370	230	346	133	0.78
Pharmacogenetics					
ABCC2 -24C>T (CC:CT:TT)(n/a) ⁷⁾	(6:1:0)(1)		(7:3:1)(0)		-

1) Nabumetone, celecoxib or diclofenac
2) Lisinopril or enalapril
3) Amlodipine, nifedipine XL or nicardipine
4) Lansoprazole or ranitidine
5) Fisher's Exact Test
6) Mann-Whitney U-test
7) Not available