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Effect of Ganciclovir on Pharmacokinetics of Mycophenolic Mofetil, in Kidney Transplant Patients

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Abstract

Objective
Mycophenolate mofetil (MMF) is commonly administered concomitantly with ganciclovir for managing transplant recipients who infected with CMV. This study was conducted to evaluate the probable effects of ganciclovir on Mycophenolic acid (MPA) pharmacokinetic.

Materials and Methods
Ten kidney transplant recipients who full field inclusion and exclusion criterias enrolled in this study. The first full profile blood sampling was taken during the combination therapy of gancyclovir and MMF. The second sampling was taken one week after discontinuation of gancyclovir. Serum concentrations of MPA and its glucuronide metabolite (MPAG) were determined by high-performance liquid chromatography (HPLC) method. The pharmacokinetic parameters of MPA were measured, in two conditions, for each patient.

Results
There was no significant difference between MPA clearance alone and in combination with ganciclovir (28.2 ± 21.9 L/h vs 31.9 ± 21.3 L/h, p=0.207) and also no significant difference was seen between the MPA Area Under the Curve (AUC) in two conditions (43.48 ± 16.27 µg/ml.h vs 39.80 ± 20.18 µg/ml.h, p=0.221). MPAG AUC was increased significantly when the drugs were administrated in combination (957.8 ± 675.2 µg/ml.h vs 1348.6 ± 1095.1 µg/ml.h, p=0.036). Also ganciclovir induced entrohepatic recirculation of MPA in two patients.

Conclusion
The pharmacokinetic parameter of MPA was not affected by ganciclovir. But ganciclovir increased MPAG AUC and induced enterohepatic recirculation of MPA.

Keywords: Clearance, Kidney Transplantation, Ganciclovir, Mycophenolic acid

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Introduction

Mycophenolic acid (MPA), the active metabolite of prodrug mycophenolate mofetil (MMF), exerts its immunosuppressive action by blocking T and B-cell proliferation via inhibiting inosin monophosphate dehydrogenase (IMPDH) which is a key enzyme in *de novo* synthesis of gouanosine monophosphate (1, 2). Recent studies demonstrated its efficacy in treatment of acute rejection by interfering with the glycosylation of adhesion molecules, which have a rule in recruitment of lymphocytes in the sites of inflammation and graft rejection (3). The agent is routinely administrated at many transplant centers as standard immunosuppression therapy. Mycophenolic acid is metabolized to inactive metabolite mycophenolate glucuronide (MPAG) by glucuronyl transferases in kidney and liver. A secondary peak concentration often observed due to entrohepatic recirculation as MPAG converts to MPA by glucuronidase of gastrointestinal flora (4). Alternations in entrohepatic recirculation have a rule in MPA inter and intra individual variability (5, 6). There is a relationship between MPA area under the concentration-time curve (AUC) and risk of acute rejection in both adult and pediatric patients during early post transplant period (4, 7, 8). Cytomegalovirus (CMV) continues to be a common cause of morbidity and mortality in transplant recipients. CMV infection post-transplantation is in part influenced by degree of immunosuppression (9). Even with effective prophylactic and preemptive treatment strategies, it is the most concerning viral agent in transplant recipients (10). Ganciclovir is the choice treatment for cytomegaloviral infection in immunocompromised patients such as transplant recipients (11). Mycophenolate will undoubtedly be administered concomitantly with ganciclovir for managing transplant recipients who infected with CMV. Ganciclovir is eliminated primarily by the kidneys by glomerular filtration and tubular secretion (12-14). Since MPAG and ganciclovir are both excreted by the kidneys, competition for renal elimination may occur, although this is not necessarily expected.

Unfortunately, there is little information available about the effect of ganciclovir on pharmacokinetics of Mycophenolic Mofetil and its glucuronide metabolite and the results of this field are controversial. Therefore, in this study, the probable effects of ganciclovir on MPA pharmacokinetic were evaluated.

Materials and Methods

Patients

This study was carried out prospectively between June 2005 and September 2006, on ten renal transplant recipients at transplantation center of Imam-Reza Hospital in Mashhad, Iran. This study was approved by the local ethics committee of MUMS. The Patients fulfilled inclusion and exclusion criteria as follows:

Patients were included if:

1. They received immunosuppressive therapy consisting of the fix dose of MMF (1 g, bid), standard dose of cyclosporine and prednisolone
2. Their MPA blood level was achieved to steady state.
3. They infected by CMV and received ganciclovir.
4. Their liver and kidney function were normal.

Patients were excluded if:

1. They received polyclonal antibody.
2. They suffered from acute rejection.

Blood sampling and drug assay

At completion of the screening period, patients who infected with CMV, received ganciclovir (10 mg/kg/day) and MMF (2g/day) concomitantly. The patients received combination therapy for seven days, then their blood samples were taken at 0 (predose), 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 7, 8, 9, 10 and 12 hours after administration of the MMF dose. When the ganciclovir therapy was completed, it was discontinued and then after a 7-days wash out period, a full profile blood samplings, as mentioned above, were taken from patients again. Plasma samples removed immediately after centrifugation (10 min, 10000 g) and stored at -70° C until they were analyzed. Plasma MPA levels determined by

high-performance liquid chromatography (HPLC) method. HPLC system consisted of a double reciprocating pump (Shimadzu, LC-10ADVP). The mobile phase included a mixture of aqueous solution of potassium dihydrogen phosphate and tetra n-butyl ammonium hydrogen sulfate (20 mM, 40 mM), acetonitrile (33:67 v/v) with a final pH of 5.5 and pumped at 0.8 ml/min. The stationary phase was Eurosphere 100 C_{18} column (125×4 mm ID, 5μm) used for separation at room temperature, connected to a guard column packed with the same bonded phase (5×4mm ID). The detection made by a UV detector (Shimadzu SPD-10 AVD) at wavelength of 254 nm. Chromatography data collected and processed on Eurocrome software. The results of chromatographic method validation were mean absolute recovery (96.3 %) for MPA and (101%) for MPAG, limit of quantitation was 0.1 mg/ l for MPA and 2.5 mg/l for MPAG: Within day reproducibility and between days reproducibility were less than 10% for MPA plasma concentration 0.5, 1, 5, 10, 25, 40 mg/l and for MPAG plasma concentration 10, 25, 50, 200, 300, 400 mg/l. The calibration curve obtained over the concentration range of 0.5-40 mg/l. The r^2 value was 0.9999 for MPA (AUC=18309C+41670) and achieved over the concentration range of 10-400 mg/l and r^2 value was 0.9996 for MPAG (AUC=15154C-54239).

Pharmacokinetics and statistical analysis
The area under the plasma or serum concentration-time curve (AUC) was calculated by the linear trapezoidal rule, according to equation (1).

$$AUC_{(0-12)} = \Sigma_{i=1}^{n} \frac{(C_i + C_{i+1})}{2}(t_{i+1} - t_i) \qquad \text{(Equation 1)}$$

The maximum concentrations ($Cmax_1$, $Cmax_2$) and maximum times ($tmax_1$, $tmax_2$) were the observed values. Apparent MPA plasma clearance (CL) calculated by equation (2).

$$\frac{CL}{F} = \frac{Dose}{AUC} \qquad \text{(Equation 2)}$$

All statistical analysis performed using SPSS software for windows (version 11.5 USA). Paired t test was used, between two groups, for comparison of pharmacokinetics parameters. p value less than 0.05 considered significant.

Results
Population study
This study was enrolled for 10 patients, age 16 to 51 years (mean age 32.67± 11.67 years; 5 women, 5 men). Characteristics of patients are summarized in table 1.

Table 1. Characteristic of patients

Number of patients	10
Age (years)	32.67±11.6[1]
Sex ratio (F/M)	1
TBW (kg)	50.58±8.27[1]
Serum creatinine (mg/dl)	1.91±1.58[1]
ALT (U/L)	41.75±62.46[1]
AST(U/L)	30.33±28.15[1]
Time after Transplantation (days)	368.33±586.07[1]
Donor status (cadavor/living donor)	6/4
Mycophenolate mofetil dose (mg/day)	2000
Prednisolone dose (mg/day)	27.78[2] (8-35)[3]
Cyclosporine dose (mg/day)	230[2] (200-300)[3]

1. Mean±SD, 2.mean, 3.range

Comparison of pharmacokinetic parameters of MPA when MMF administrated alone and in combination with ganciclovir
The results of pharmacokinetic parameters of MPA when MMF administrated alone and in combination with ganciclovir are summarized in table 2. As it is shown in table 2, there was no significant difference between MPA AUC, clearance, $Cmax_1$, $Cmax_2$, $Tmax_1$, $Tmax_2$ values, when MMF administrated alone and in combination with ganciclovir. Figures 1, 2 illustrate mean plasma concentration versus time profile for MPA after administration of MMF alone and in combination with ganciclovir respectively.

Table 2. The comparison of pharmacokinetic parameters of MPA when MMF administrated alone and with ganciclovir

Parameter's pharmacokinetic	Without ganciclovir	With ganciclovir	p-Value[2]
AUC (µg/ml.h)	43.48±16.27[1]	39.80±20.18[1]	0.207
CL (L/h)	28.22±21.91[1]	31.93±21.29[1]	0.221
$Cmax_1$ (µg/ml)	12.11±14.21[1]	14.10±11.82[1]	0.132
$Cmax_2$ (µg/ml)	5.19± 6.06[1]	2.25±1.46[1]	0.161
$Tmax_1$ (h)	1.84±0.99[1]	1.98 1.26[1]	0.708
$Tmax_2$ (h)	9 ±0.81[1]	8.45 ±0.83[1]	0.247

1.Mean±SD, 2.Paired T test
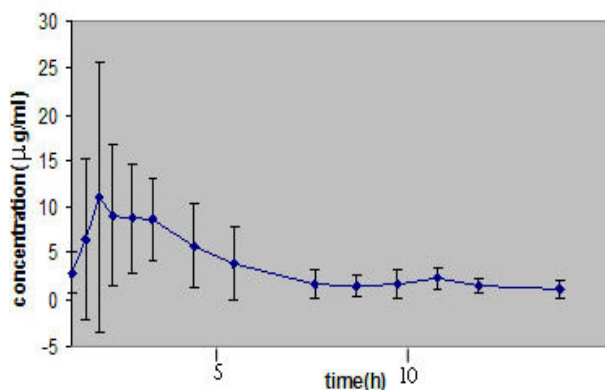
Figure 1. Mean plasma concentration versus time profiles for MPA when MMF administrated alone.
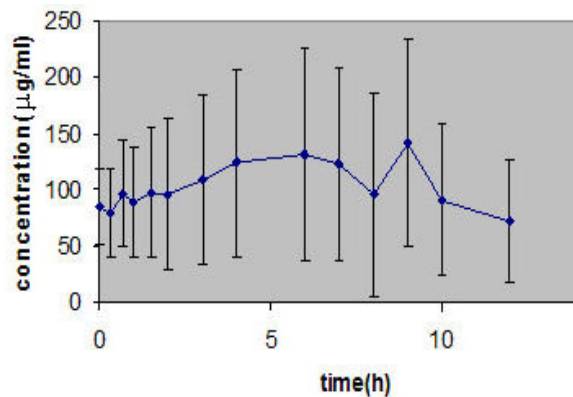

Figure 3. Mean plasma concentration versus time profiles for MPAG when MMF administrated alone.
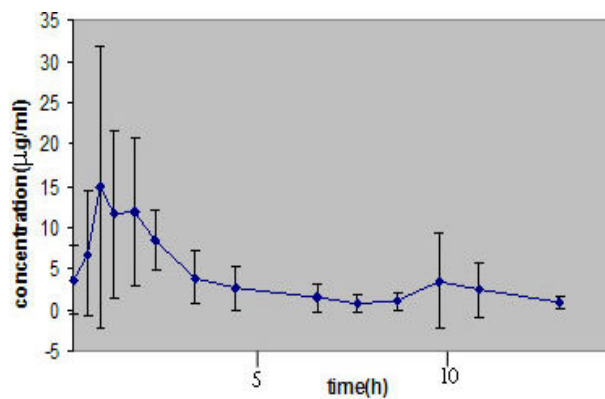

Figure 2. Mean plasma concentration versus time profiles for MPA when MMF administrated with ganciclovir.
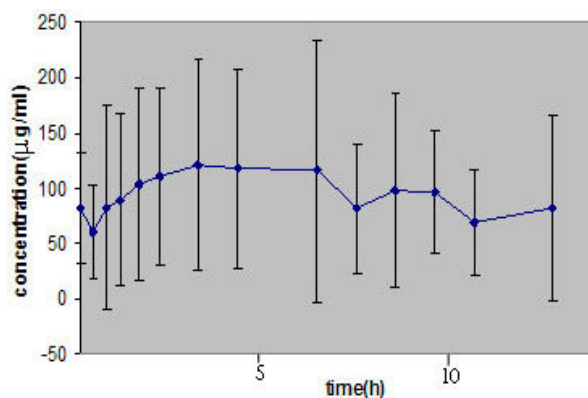

Figure 4. Mean plasma concentration versus time profiles for MPAG when MMF administrated in combination with ganciclovir

Comparison of MPAG AUC in two different conditions

The results of MPAG AUC when MMF administrated alone and in combination with ganciclovir are shownd in table 3. As it is indicated in table 3, MPAG AUC was increased significantly when the drugs administrated in combination.

Figures 3 and 4 illustrate mean plasma concentration versus time profile for MPAG after MMF administration, alone and in combination with ganciclovir respectively.

Table 3. The comparison of AUC of MPAG when MMF administrated alone and with ganciclovir.

MPAG	Without ganciclovir	With ganciclovir	p-value
AUC (µg/ml.h)	675.18[1] ±957.83	1095.14[1]±1348.63	0.036[2]

1. Mean±SD, 2. Paired T test

Discussion

MMF is commonly administered concomitantly with ganciclovir for managing transplant recipients who infected with CMV. It is necessary to evaluate MPA and MPAG interaction with ganciclovir in this condition. There are several animal studies concerning this interaction. A single-dose crossover animal study performed on four cynomolgus monkeys, which demonstrated a 2.4-fold increase in plasma levels of MPA when administered with ganciclovir. In another animal study when MMF administrated with ganciclovir a similar increase (2.2-fold) was seen for MPAG (15). Unfortunately despite the result of animal study about potential interaction of these drugs, only one human study was performed in this field. Wolf et al carried out a randomized, open-lable, three-way cross over study on 12 kidney transplant patients for evaluation of possible drug interaction between MPA and ganciclovir (14). This study reported that the single dose pharmacokinetics of MPA and its glucuronide metabolite were unchanged when MMF administrated with ganciclovir. Base on these studies, the effect of ganciclovir on pharmacokinetic of MPA is controversial. Therefore more studies are needed. In the present study the possible drug interaction between MPA and ganciclovir in adult transplant recipients was evaluated.

The results demonstrated that during concurrent therapy of MMF and ganciclovir, MPA AUC was unaltered while MPAG AUC increased significantly. Shab et al reported that the renal clearance of MPAG correlated by simple linear regression with creatinine clearance, therefore it would be expected that MPAG is eliminated primarily by the kidneys (16). Other investigators reported that accumulation of MPAG may occur in renal impairment. Ganciclovir is eliminated primarily unchanged by glomerular filtration and tubular secretion. Therefore, it is suggested that possible competition between ganciclovir and MPAG for active renal tubular secretion cause a significant increase in MPAG AUC when ganciclovir co administrated with MMF (15).

Also this study showed that ganciclovir induce second peak of MPA (Cmax2) in two patients. The concentration-time data for MPA suggested bimodel disposition consistent with enterohepatic recirculation of drug eliminated in bile. Therefore, MPAG is converted back to MPA in the small intestine and reabsorbed, resulting in the second plasma peak (14, 15). The conversion of MPA to the pharmacologically inactive phenolic acid glucuronide (MPAG) is catalyzed by UDP-glucuronosyltransferase. The most likely sites for this conversion are in the gastrointestinal tract, liver and possibly kidney. This metabolic step is generally regarded as quantitatively the most important and rate limiting one (5). It is reported that a few drugs such as cyclosporine, prednisolone and tacrolimus interact with entrohepatic recirculation of MPA through induction or inhibition of UDP-glucuronosyltransferase (18-20). Therefore, it is suggested that ganciclovir may induce entrohepatic recirculation of MPA in these two patients.

Acknowledgement

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