Published in final edited form as:

Xenobiotica. 2013 March ; 43(3): 229–235. doi:10.3109/00498254.2012.713531.

Mycophenolic Acid Glucuronide (MPAG) is Transported by Multidrug Resistance-Associated Protein 2 (MRP2) and this Transport is not Inhibited by Cyclosporine, Tacrolimus or Sirolimus

Chirag G. Patel^{1,2,#}, Ken Ogasawara^{1,#}, and Fatemeh Akhlaghi¹

¹Clinical Pharmacokinetics Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI, USA

Abstract

1. The purpose of this study was to investigate the contribution of MRP2 to the efflux of mycophenolic acid (MPA), and its phenyl glucuronide (MPAG) and acyl glucuronide (AcMPAG) metabolites, using Madin-Darby canine kidney II cells stably transfected with human *MRP2* gene (MDCKII/MRP2 cells).
2. Compared to parental MDCKII cells, MPAG was significantly translocated from basolateral (BL) to apical (AP) side in MDCKII/MRP2 cells, indicating MPAG is a substrate for MRP2. AcMPAG is highly translocated from BL to AP side in both cells, suggesting that AcMPAG is actively secreted possibly through an efflux transporter other than MRP2. Appreciable translocation of MPA was not observed in MDCKII/MRP2 cells.
3. Furthermore, using MRP2-expressing Sf9 membrane vesicles, the Michaelis-Menten constant (Km) value for MRP2-mediated MPAG transport was calculated at $224.2 \pm 42.7 \mu\text{M}$. In the vesicle system, cyclosporine, tacrolimus and sirolimus did not inhibit the uptake of MPAG via MRP2.
4. These findings indicate that only MPAG not MPA and AcMPAG is a substrate for MRP2 and that the interaction between MPAG and concomitantly administered immunosuppressive agents does not occur at MRP2 level.

Keywords

AcMPAG; drug interactions; immunosuppressive agents; MDCK-MRP2; MPA; MPAG; pharmacokinetics; translocation

Corresponding author and address, Fatemeh Akhlaghi, PhD, Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, 125 Fogarty Hall, 41 Lower College Road, Kingston, RI 02881, USA, Phone: (401) 874 9205, Fax: (401) 874 5787, fatemeh@uri.edu.

²Current address, Millennium Pharmaceuticals, Inc., Cambridge, MA, USA

[#]These two authors contributed equally to the work.

Declaration of Interest: The authors have no conflicts of interest to report. The authors alone are responsible for the content and writing of this manuscript.

Introduction

Mycophenolic acid (MPA) is an antiproliferative agent commonly used for the prevention of organ transplant rejection or the treatment of autoimmune diseases. In transplant recipients, it is administered in combination with cyclosporine (CsA), tacrolimus (TAC) or sirolimus (SIR) and with a corticosteroid such as prednisone (Halloran, 2004). The major adverse effect of MPA is gastrointestinal toxicity possibly related to prolonged exposure to MPA or metabolites in the gastrointestinal tract (Davies et al., 2007; Neerman and Boothe, 2003; Nguyen et al., 2009). Understanding the underlying mechanisms governing metabolism and transport of MPA and its metabolites are warranted to allow optimized immunotherapy with this medication.

Mycophenolic acid is metabolized through phase 2 conjugation by the uridine diphosphate glucuronosyl transferase (UGT) system to phenyl mycophenolic acid glucuronide (MPAG) and acyl mycophenolic acid glucuronide (AcMPAG) (Picard and Marquet, 2004). AcMPAG is believed to be pharmacologically active, having similar functions as MPA (Shipkova et al., 2002). MPAG is the major metabolite of MPA with MPAG/MPA maximum concentration ratio of ~10 and MPAG/MPA area under the concentration-time curves (AUC) ratio of ~100 (Kuypers et al., 2003). Both MPAG and AcMPAG are primarily eliminated by the renal route, whereas MPAG also undergoes enterohepatic recirculation via biliary secretion (Shaw et al., 1998). Enterohepatic recycling of MPAG to MPA extend the presence of MPA in the gastrointestinal tract resulting in toxicity and increases the overall exposure to MPA.

Transport of MPA and its metabolites by various transporters has been elucidated. Organic anion transporting polypeptide (OATP) 1B3 and to a lesser extent OATP1B1 are believed to be involved in the uptake of MPAG into hepatocytes (Picard et al., 2010). Furthermore, *in vitro* studies demonstrated that organic anion transporter (OAT) 1 and OAT3 are inhibited by MPA and metabolites while OAT3 may also contribute to renal tubular secretion of MPAG (Wolff et al., 2007). As for the efflux transporters, several studies in multidrug resistance-associated protein 2 (Mrp2) deficient rats have indicated a critical role for MRP2 transport on the pharmacokinetics of MPA and its metabolites (Hesselink et al., 2005; Kobayashi et al., 2004; Westley et al., 2006). In addition, clinical studies have showed that *MRP2* -24C>T affected the pharmacokinetics of MPA and its metabolites (Lévesque et al., 2008; Lloberas et al., 2011; Naesens et al., 2006). MRP2, which is a canalicular transporter that is localized in the apical side of cells in the liver, kidney and the small intestine, plays a crucial role in exporting amphiphilic organic anions from the hepatocytes into bile or from cells into the lumen of excretory organs including kidneys (Ho and Kim, 2005). However, MRP2-mediated transport of MPA and its metabolites has never been demonstrated by *in vitro* studies and it was not known whether they are substrates for human MRP2.

Because MPA, MPAG and AcMPAG are organic anions, we hypothesized that all three compounds were substrate for human MRP2. Therefore, we have studied their MRP2-mediated transport using Madin-Darby canine kidney II cells (MDCKII), stably transfected with human *MRP2* gene (MDCKII/MRP2 cells) (Evers et al., 1998). In addition, the

interaction between MPA or its metabolites and concomitantly used medications on MRP2 was investigated.

Materials and Methods

Materials

MK-571 sodium salt was purchased from Axxora LLC (San Diego, CA), TAC and SIR from LC laboratories (Woburn, MA), CsA and 5(6)-carboxy-2', 7'-dichlorofluorescein (CDCF) from Sigma (St. Louis, MO). MPA, MPAG and AcMPAG were kindly donated by Roche Laboratories (Nutley, NJ). All other chemicals and reagents were purchased from either Fischer Scientific (Pittsburgh, PA) or Invitrogen (Carlsbad, CA), as required.

Cell Culture

MDCKII/MRP2 cells and MDCKII cells transfected only with the expression vector (MDCKII/Par) were kindly provided by Dr. Piet Borst (The Netherlands Cancer Institute, Amsterdam, Netherlands). The MDCKII cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, and 2% penicillin-streptomycin in a 37°C, 5% CO₂ humidified incubator. The cells were then seeded onto transwell polyethylene filter inserts at 665000 cells/cm² density and grown for 3 to 4 days. To confirm the formation of a confluent monolayer on the inserts, Lucifer yellow diffusion test (100 µM concentration per well added to the apical side) was performed on selected wells. The transepithelial electrical resistance (TEER) was measured on every well before the diffusion experiments by the use of a Millicell ERS[®] voltohmmeter (Millipore, Bedford, MA). Resistance values of >150 Ω cm² after subtracting the resistance from a blank transwell (no cellular monolayer) were indicative of the formation of a cell monolayer with tight junctions.

Diffusion Studies in MRP2 Transfected and Parental Cells

Diffusion studies were carried out in both MDCKII/MRP2 and MDCKII/Par cells. Basolateral (BL) to apical (AP) and AP to BL diffusions for each analyte were investigated at two concentrations, representing low and high clinically relevant levels of each analyte, MPA (1 and 10 mg/L corresponding to 3.12 and 31.2 µM, respectively), MPAG (5 and 50 mg/L corresponding to 9.27 and 92.7 µM, respectively) and AcMPAG (1 and 5 mg/L corresponding to 1.85 and 9.27 µM, respectively). After confirming the formation of the monolayer by the TEER method, the cells were washed with HBSS and the experiment was performed in transport buffer (HBSS with 1% dimethyl sulfoxide). The transwell plates were incubated in a humidified cell culture incubator and samples were drawn at 30, 60, 90, 120, 150 and 180 minutes from AP and BL side. The flux of the analytes was first calculated after determining the concentration of analyte using Equation 1. The apparent permeability (P_{app}) (BL to AP) was then determined using Equation 2. Similarly, P_{app} (AP to BL) was estimated using Equation 2 but with AP to BL flux value. Finally, the polarization ratio was determined using Equation 3 and an efflux ratio ≥ 2 indicated that transport is apically polarized (active efflux) (Giacomini et al., 2010). The analyte with an efflux ratio at least 2 times greater for MDCKII/MRP2 than that of MDCKII/Par cells was considered to be a substrate for MRP2.

$$Flux(F) = \frac{\text{Concentration of analyte in receiver chamber (mg/L)}}{\text{Concentration of analyte in donor chamber at } t=0 \text{ (mg/L)}} \quad \text{Eq. 1}$$

$$P_{app}(BL \text{ to } AP) = \frac{F(BL \text{ to } AP) \times 0.7 \text{ cm}^3 (\text{volume of donar chamber})}{\text{Duration of incubation (minutes)} \times 0.3 \text{ cm}^2 (\text{area of cell layer})} \quad \text{Eq. 2}$$

$$Efflux \text{ ratio} = \frac{P_{app}(BL \text{ to } AP) (\text{cm/min})}{P_{app}(AP \text{ to } BL) (\text{cm/min})} \quad \text{Eq. 3}$$

Diffusion Studies with Inhibitors

As the positive control for the experiments, MK-571, a known MRP2 inhibitor ($K_i \sim 10 \mu\text{M}$), was used at $50 \mu\text{M}$ and $100 \mu\text{M}$, which completely inhibit MRP2-mediated transport. Solutions of MPAG and interacting agents (CsA, TAC, SIR) were prepared in HBSS at two levels, roughly representing low and high clinically relevant unbound (free) concentration, for an interacting agent, CsA (1 and $10 \mu\text{g/L}$ corresponding to 0.83 and 8.3 nM , respectively), TAC (0.1 and $1 \mu\text{g/L}$ corresponding to 0.124 and 1.24 nM , respectively) and SIR (0.1 and $0.5 \mu\text{g/L}$ corresponding to 0.109 and 0.546 nM , respectively) (Goodman et al., 2006). Solutions of the interacting agents were placed on both BL and AP sides during cell culture. On the day of the experiment, TEER was measured and the inserts were washed with HBSS. The solution of the interacting agents, along with MPAG, was then added to both sides.

Transport Studies by Membrane Vesicles

The vesicular transport study using human MRP2-expressing Sf9 membrane vesicles (GenoMembrane Inc., Yokohama, Japan) was carried out by a rapid filtration technique (Yamaguchi et al., 2010) with a slight modification. Briefly, the incubation mixture contained appropriate amount of MPAG or $5 \mu\text{M}$ CDCF, 4 mM ATP or AMP, $15 \mu\text{g}$ of membrane vesicles in $50 \mu\text{L}$ of the transport buffer (50 mM MOPS–Tris buffer, pH 7.0, containing 70 mM KCl and 7.5 mM MgCl_2), was incubated in a test tube for 5 min at 37°C . The incubation was terminated by diluting the reaction mixture with 1 mL of ice-cold stop solution (40 mM MOPS–Tris buffer, pH 7.0, containing 70 mM KCl). The mixture was poured immediately onto Millipore filters (HAWP, $0.45 \mu\text{m}$, 4.7 cm in diameter), and the filters were washed with 5 mL of ice-cold stop solution. Drugs trapped in vesicles retained on the filter was eluted with $50:50$ acetonitrile:water (0.4 mL). Fluorescence intensity of CDCF was measured by SpectraMax M2 microplate reader (Molecular Devices, Downingtown, PA). Concentration of MPAG was determined by an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method. ATP-dependent transport activity was calculated by subtracting the transport activity in the presence of AMP from that in the presence of ATP. Using nonlinear regression analysis, the Michaelis-Menten constant (K_m) and the maximum uptake rate (V_{max}) were calculated with the following equation, $V = V_{\text{max}} \times [S]/(K_m + [S])$, where V is ATP-dependent uptake amount, $[S]$ is the concentration of MPAG.

Sample Preparation and HPLC Condition

50 μ L of the sample of diffusion studies was mixed with 100 μ L combined internal standard solution and 100 μ L of 0.1 % (v/v) phosphoric acid. Combined internal standard solution containing 200 mg/L phenolphthalein glucuronic acid (internal standard for MPAG) and 20 mg/L carboxy butoxy ether of MPA (internal standard for MPA and AcMPAG) was prepared in methanol. An aliquot of 50 μ L of either calibrators or unknown in diffusion buffer was injected onto the analytical column. An in-house developed and validated high-performance liquid chromatography (HPLC)-UV analytical method was used for quantification of MPA and its metabolites (Patel and Akhlaghi, 2006). The lower limit of quantification (LLOQ) for MPA, MPAG and AcMPAG were 0.2, 2 and 0.5 mg/L, respectively. The accuracy and precision of the assay was better than $\pm 15\%$.

UPLC-MS/MS

The chromatographic separation was achieved with an Acquity UPLC BEH C18 column, 2.1×50 mm, 1.7μ m (Waters Corp., Milford, MA) maintained at 55°C . The mobile phase that consisted of (A) 2 mM ammonium acetate with 0.1% v/v formic acid and (B) acetonitrile with 1 mM ammonium acetate was pumped at a flow rate of 0.4 mL/min. The following gradient elution mode was used: for the first 0.5 minutes of each run, the mobile phase remained at A:B 95:5 (v/v), from 0.5 to 1.4 minutes there was a continuous gradient change to A:B 2:98 (v/v), and from 1.4 to 2.4 minutes the composition maintained at A:B 2:98 (v/v). After 2.4 minutes, the composition changed back to A:B 95:5 (v/v) with a fast gradient method (within 6 seconds), followed by a re-equilibration until 3.0 minutes. Mass spectrometry detection of positive ions in multiple reaction monitoring was performed using Xevo TQ MS (Waters Corp.) equipped with an electrospray ionization source (ESI). The desolvation gas flow rate was set to 1000 L/h at a temperature of 500°C , the cone gas flow rate was set at 50 L/h and the source temperature at 150°C . The capillary and cone voltage was set to 1400 V and 14 V, respectively. The mass transition for the ammonium adduct of MPAG was m/z 514.3 to 303.2 with retention time 1.17 minutes. The LLOQ for MPAG quantification 0.3 nM or 0.15 μ g/L and the accuracy and precision of the assay was better than $\pm 10\%$.

Results

Diffusion Studies of MPA, MPAG and AcMPAG

Firstly, diffusion studies were carried out at two concentrations for MPA, MPAG and AcMPAG in MDCKII/MRP2 and MDCKII/Par cells. Apparent permeability values for unidirectional translocation of each analyte and the corresponding efflux ratio are shown in Table 1. In addition, efflux ratios of MPA (Figure 1A), MPAG (Figure 1B) and AcMPAG (Figure 1C) in MDCKII/MRP2 cells in comparison with efflux ratios observed using MDCKII/Par cells are depicted. Transport studies with MPA in MDCKII/MRP2 cells yielded an efflux ratio <2 and similar efflux ratios were observed in MDCKII/Par cells (Figure 1A), indicating that MPA is not a substrate for MRP2. Based on the values of efflux ratios, the translocation of MPAG from BL to AP side (efflux) for MDCKII/MRP2 cells was almost 6 times greater than its translocation from the AP to BL side (influx). Moreover, in comparison to the MDCKII/Par cells, efflux ratios for MPAG were 2-3 folds greater in the

MDCKII/MRP2 cells (Figure 1B). These results indicate that MPAG is a substrate for MRP2. A comparison for AcMPAG in MDCKII/MRP2 revealed the efflux was 9 times greater than the corresponding influx. However, similar efflux was observed in MDCKII/Par cells, indicating AcMPAG is actively secreted possibly through a transporter other than MRP2 that is also expressed in this canine kidney cell line (Figure 1C).

Diffusion Studies with Inhibitors

Subsequent *in vitro* drug interaction experiments were only performed with MPAG since MPA and AcMPAG were not found to be substrates for MRP2. Moreover, a sampling time point of 180 min was selected for inhibition experiments since the rate of active efflux of MPAG had reached a plateau at this time point (Figure 1B). The interaction studies in MDCK/MRP2 cells were then carried out for MPAG transport using immunosuppressive agents that are commonly co-administered with MPA. As depicted in Figure 2, at clinically relevant concentrations, CsA produced the strongest apparent inhibition of MPAG transport, followed by SIR and TAC. Neither SIR nor TAC inhibited MPAG translocation at the lower end of clinically relevant concentrations.

Transport Studies by Membrane Vesicles

MRP2-expressing membrane vesicles were used to evaluate the effects of the immunosuppressive agents on MRP2-mediated MPAG transport. Firstly, concentration dependence of ATP-dependent uptake of MPAG was studied. Kinetic analysis demonstrated that ATP-dependent uptake of MPAG by MRP2-expressing membrane vesicles was saturable with a $V_{\max}$ value of 2.7 ± 0.2 nmol/mg protein/5 min and a K_m value of 224.2 ± 42.7 μ M (Figure 3A). Next, the inhibitory effects of CsA, TAC and SIR on MPAG uptake by MRP2-expressing membrane vesicles were investigated at the higher end of clinically relevant unbound concentration, which sufficiently inhibited the translocation of MPAG in MDCK/MRP2 cells (Figure 2). In contrast to the interaction studies in MDCK/MRP2 cells, CsA, TAC and SIR had no inhibitory effects on the MPAG uptake by MRP2-expressing membrane vesicles (Figure 3B). As shown in Figure 3C, we also confirmed that none of the immunosuppressive agents included in the study, inhibited the transport of CDCF, a known MRP2 substrate in MRP2-expressing membrane vesicles.

Discussion

In the present study, we have described the results of an *in vitro* investigation into the translocation of MPA and metabolites, MPAG and AcMPAG, by human MRP2. Mycophenolic acid has complex pharmacokinetic characteristics including a secondary peak in its concentration-time profile because of enterohepatic recirculation of MPAG. Enterohepatic recycling is an important component of MPA pharmacokinetics because it contributes to a sizable fraction of MPA systemic exposure thus any interference with this pathway may significantly alter MPA disposition. In this study, we have demonstrated that MPAG is a substrate for MRP2 using MDCK/MRP2 cells and MRP2-expressing membrane vesicles. These results indicate that MRP2 mediated transport is a particularly important mechanism governing MPAG disposition and considering the tissue expression of MRP2, it can be speculated the MRP2 affect both enterohepatic recirculation and renal excretion of

MPAG. In contrast, our results indicate that MPA is not a substrate for active efflux by MRP2. AcMPAG had an efflux ratio of ~ 9 although similar ratios were observed between MRP2 transfected and the parental cells. This suggests that AcMPAG is actively secreted through not MRP2 but an unknown transporter that is expressed in the apical membrane of MDCK cell line.

The interaction studies in MDCK/MRP2 cells were then carried out for MPAG transport using drugs that are commonly co-administered with MPA. We observed that at clinically relevant concentrations, CsA, TAC and SIR inhibited MPAG transport in MDCK/MRP2 cells. MPAG has a low permeability and the transport of MPAG from basolateral side into apical side needs the uptake transporters at the basolateral membrane as well as MRP2 at the apical membrane. Therefore, to clarify the impact of these immunosuppressants on the transport of MPAG by only MRP2, MRP2-expressing membrane vesicles were used. We demonstrated that ATP-dependent uptake of MPAG by MRP2-expressing membrane vesicles was saturable with a K_m of $224.2 \pm 42.7 \mu\text{M}$. Takekuma et al. (2007) reported that MPAG inhibited the uptake of p-aminohippuric acid via MRP2 with the IC_{50} value of $1036.8 \pm 330.5 \mu\text{M}$, which is comparable to the K_m calculated in the present study.

In contrast to the interaction studies in MDCK/MRP2 cells, CsA, TAC and SIR had no inhibitory effects on the MPAG uptake by MRP2-expressing membrane vesicles. These contradicting findings can be originated from the fact that CsA, TAC and SIR reduced MPAG transport in MDCK/MRP2 cells via the inhibition of uptake transporters at basolateral membrane but not MRP2. Although uptake transporters natively expressed in MDCK cells are not identified, MPAG is reported to be a substrate of the human liver-specific uptake transporter OATP1B3 and, to a lesser extent, OATP1B1. Moreover, an *OATP1B3* common polymorphism 334T>G influences the pharmacokinetics of MPA and MPAG in renal transplant patients (Picard et al., 2010). In addition, CsA, TAC and SIR have been reported to inhibit OATP1B1 and OATP1B3 systems (Kalliokoski and Niemi, 2009; Picard et al., 2011). Several studies in *Mrp2* deficient rats (Hesselink et al., 2005; Kobayashi et al., 2004; Westley et al., 2006) showed that CsA inhibited the biliary excretion of MPAG and suggested that this interaction occurred at the MRP2 level. However, our results suggest that the inhibition of biliary excretion of MPAG by CsA could be due to the inhibition of uptake transporters rather than MRP2. Further studies are needed to elucidate the mechanisms underlying the interaction between MPAG and CsA.

The present study is the first study to evaluate the disposition of MPA and its glucuronide metabolites using a cell line and vesicles expressing human MRP2 since all previous studies have used *Mrp2* deficient rodents. Although *Mrp2* deficient rat is a convenient model for studying the possibility of drug interaction, a 200% increase in the expression of *Mrp4* was observed in the kidneys of these rats (Chen et al., 2005; Chen et al., 2008). If a drug is a substrate for both *Mrp2* and *Mrp4*, the data obtained in *Mrp2* deficient rat can be misleading and may lack clarity about specific interactions. In addition, species specific differences was observed in MRP2-mediated transport kinetics of estradiol-17 β -D-glucuronide between rat and human that questions the validity of rat *Mrp2* as a model for human MRP2 transport (Heredi-Szabo et al., 2009). Moreover, our results suggest that MDCK/MRP2 cell is not an

appropriate model to evaluate the possibility of drug interactions with a polar compound like MPAG at MRP2 level.

Conclusions

In summary, this is the first study to demonstrate that MPAG is a substrate for human MRP2. In addition, MPAG transport via MRP2 was not inhibited by CsA, TAC and SIR, indicating that clinically observed drug-drug interactions with MPA is not due to the inhibition of MRP2-mediated efflux of MPAG by these agents. Further studies are needed to characterize the specific transporters responsible for the translocation of AcMPAG.

Acknowledgments

We thank Dr. Ruitang Deng (University of Rhode Island) for many helpful discussions about experimental design. Partial support of grant # R15 GM101599-01 from National Institutes of Health is gratefully acknowledged. This research was made possible by the use of the RI-INBRE Research Core Facility, supported jointly by NCRR/NIH Grant # P20 RR016457 and the network institutions.

References

- Chen C, Slitt AL, Dieter MZ, Tanaka Y, Scheffer GL, Klaassen CD. Up-regulation of Mrp4 expression in kidney of Mrp2-deficient TR- rats. *Biochem Pharmacol.* 2005; 70:1088–95. [PubMed: 16099435]
- Chen J, Terada T, Ogasawara K, Katsura T, Inui K. Adaptive responses of renal organic anion transporter 3 (OAT3) during cholestasis. *Am J Physiol Renal Physiol.* 2008; 295:F247–52. [PubMed: 18480179]
- Davies NM, Grinyo J, Heading R, Maes B, Meier-Kriesche HU, Oellerich M. Gastrointestinal side effects of mycophenolic acid in renal transplant patients: a reappraisal. *Nephrol Dial Transplant.* 2007; 22:2440–8. [PubMed: 17557774]
- Evers R, Kool M, van Deemter L, Janssen H, Calafat J, Oomen LC, Paulusma CC, Oude Elferink RP, Baas F, Schinkel AH, Borst P. Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA. *J Clin Invest.* 1998; 101:1310–9. [PubMed: 9525973]
- Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, Dahlin A, Evers R, Fischer V, Hillgren KM, Hoffmaster KA, Ishikawa T, Keppler D, Kim RB, Lee CA, Niemi M, Polli JW, Sugiyama Y, Swaan PW, Ware JA, Wright SH, Yee SW, Zamek-Gliszczynski MJ, Zhang L. Membrane transporters in drug development. *Nat Rev Drug Discov.* 2010; 9:215–36. [PubMed: 20190787]
- Goodman, LS.; Gilman, A.; Brunton, LL.; Lazo, JS.; Parker, K. Goodman & Gilman's the pharmacological basis of therapeutics. 11th. New York: McGraw-Hill; 2006.
- Halloran PF. Immunosuppressive drugs for kidney transplantation. *N Engl J Med.* 2004; 351:2715–29. [PubMed: 15616206]
- Herédi-Szabó K, Glavinas H, Kis E, Méhn D, Báthori G, Veres Z, Kóbori L, von Richter O, Jemnitz K, Krajcsi P. Multidrug resistance protein 2-mediated estradiol-17 β -D-glucuronide transport potentiation: in vitro-in vivo correlation and species specificity. *Drug Metab Dispos.* 2009; 37:794–801. [PubMed: 19118132]
- Hesselink DA, van Hest RM, Mathot RA, Bonthuis F, Weimar W, de Bruin RW, van Gelder T. Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. *Am J Transplant.* 2005; 5:987–94. [PubMed: 15816878]
- Ho RH, Kim RB. Transporters and drug therapy, implications for drug disposition and disease. *Clin Pharmacol Ther.* 2005; 78:260–77. [PubMed: 16153397]
- Kalliokoski A, Niemi M. Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol.* 2009; 158:693–705. [PubMed: 19785645]

- Kobayashi M, Saitoh H, Kobayashi M, Tadano K, Takahashi Y, Hirano T. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. *J Pharmacol Exp Ther*. 2004; 309:1029–35. [PubMed: 14978191]
- Kuypers DR, Vanrenterghem Y, Squifflet JP, Mourad M, Abramowicz D, Oellerich M, Armstrong V, Shipkova M, Daems J. Twelve-month evaluation of the clinical pharmacokinetics of total and free mycophenolic acid and its glucuronide metabolites in renal allograft recipients on low dose tacrolimus in combination with mycophenolate mofetil. *Ther Drug Monit*. 2003; 25:609–22. [PubMed: 14508385]
- Levesque E, Benoit-Biancamano MO, Delage R, Couture F, Guillemette C. Pharmacokinetics of mycophenolate mofetil and its glucuronide metabolites in healthy volunteers. *Pharmacogenomics*. 2008; 9:869–79. [PubMed: 18597651]
- Lloberas N, Torras J, Cruzado JM, Andreu F, Oppenheimer F, Sanchez-Plumed J, Gentil MA, Brunet M, Ekberg H, Grinyo JM. Influence of MRP2 on MPA pharmacokinetics in renal transplant recipients-results of the Pharmacogenomic Substudy within the Symphony Study. *Nephrol Dial Transplant*. 2011; 26:3784–93. [PubMed: 21427078]
- Naesens M, Kuypers DR, Verbeke K, Vanrenterghem Y. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. *Transplantation*. 2006; 82:1074–84. [PubMed: 17060857]
- Neerman MF, Boothe DM. A possible mechanism of gastrointestinal toxicity posed by mycophenolic acid. *Pharmacol Res*. 2003; 47:523–6. [PubMed: 12742006]
- Nguyen T, Park JY, Scudiere JR, Montgomery E. Mycophenolic acid (cellcept and myofortic) induced injury of the upper GI tract. *Am J Surg Pathol*. 2009; 33:1355–63. [PubMed: 19542873]
- Patel CG, Akhlaghi F. High-performance liquid chromatography method for the determination of mycophenolic acid and its acyl and phenol glucuronide metabolites in human plasma. *Ther Drug Monit*. 2006; 28:116–22. [PubMed: 16418705]
- Picard N, Levoir L, Lamoureux F, Yee SW, Giacomini KM, Marquet P. Interaction of sirolimus and everolimus with hepatic and intestinal organic anion-transporting polypeptide transporters. *Xenobiotica*. 2011; 41:752–7. [PubMed: 21524191]
- Picard N, Marquet P. In vitro study of mycophenolic acid glucuronidation. *Drug Metab Dispos*. 2004; 32:768–73. [PubMed: 15205394]
- Picard N, Yee SW, Woillard JB, Lebranchu Y, Le Meur Y, Giacomini KM, Marquet P. The role of organic anion-transporting polypeptides and their common genetic variants in mycophenolic acid pharmacokinetics. *Clin Pharmacol Ther*. 2010; 87:100–8. [PubMed: 19890249]
- Shaw LM, Nicholls A, Hale M, Armstrong VW, Oellerich M, Yatscoff R, Morris RE, Holt DW, Venkataramanan R, Haley J, Halloran P, Ettenger R, Keown P, Morris RG. Therapeutic monitoring of mycophenolic acid. A consensus panel report. *Clin Biochem*. 1998; 31:317–22. [PubMed: 9721428]
- Shipkova M, Armstrong VW, Weber L, Niedmann PD, Wieland E, Haley J, Tonshoff B, Oellerich M. Pharmacokinetics and protein adduct formation of the pharmacologically active acyl glucuronide metabolite of mycophenolic acid in pediatric renal transplant recipients. *Ther Drug Monit*. 2002; 24:390–9. [PubMed: 12021631]
- Takekuma Y, Kakiuchi H, Yamazaki K, Miyauchi S, Kikukawa T, Kamo N, Ganapathy V, Sugawara M. Difference between pharmacokinetics of mycophenolic acid (MPA) in rats and that in humans is caused by different affinities of MRP2 to a glucuronized form. *J Pharm Pharm Sci*. 2007; 10:71–85. [PubMed: 17498396]
- Westley IS, Brogan LR, Morris RG, Evans AM, Sallustio BC. Role of Mrp2 in the hepatic disposition of mycophenolic acid and its glucuronide metabolites: effect of cyclosporine. *Drug Metab Dispos*. 2006; 34:261–6. [PubMed: 16272406]
- Wolff NA, Burckhardt BC, Burckhardt G, Oellerich M, Armstrong VW. Mycophenolic acid (MPA) and its glucuronide metabolites interact with transport systems responsible for excretion of organic anions in the basolateral membrane of the human kidney. *Nephrol Dial Transplant*. 2007; 22:2497–503. [PubMed: 17526543]

Yamaguchi K, Murai T, Yabuuchi H, Kurosawa T. Measurement of transport activities of bile acids in human multidrug resistance-associated protein 3 using liquid chromatography-tandem mass spectrometry. *Anal Sci.* 2010; 26:317–23. [PubMed: 20215680]

Figure 1.

(A) Transepithelial transport of mycophenolic acid (MPA), (B) Mycophenolic acid glucuronide (MPAG) and (C) Acyl mycophenolic acid glucuronide (AcMPAG) in a transwell monolayer of MDCKII/MDCKII/Par cells. Efflux ratios were calculated as BL to AP/AP to BL. Close symbols represent efflux ratio for each analyte in MDCKII/MDCKII/Par cells and open symbols represent data obtained from MDCKII/MDCKII/Par cells. Two concentrations for each analyte, representing low and high clinical observed levels were studied. Each data point is the average of two observations.

Figure 2.

Inhibitory effects of cyclosporine (CsA), tacrolimus (TAC) and sirolimus (SIR) on the transcellular transport of mycophenolic acid glucuronide (MPAG) across MDCKII/MRP2 cells. Efflux ratios were then calculated as BL to AP/AP to BL. MPAG concentration was 50 mg/L and the incubation time was 3 hours. Values represent mean $\pm$ SE for three replicates.

Figure 3.

(A) Concentration dependence of ATP-dependent uptake of mycophenolic acid glucuronide (MPAG) by MRP2-expressing membrane vesicles. Vesicles were incubated at 37°C for 5 min with various concentration of MPAG. ATP-dependent transport activity was calculated by subtracting the transport activity in the presence of AMP from that in the presence of ATP. Values represent mean $\pm$ SE for three replicates. (B & C) Inhibitory effects of CsA, TAC and SIR on the ATP-dependent uptake of MPAG (B) and CDCE, a specific MRP2 substrate (C) by MRP2-expressing membrane vesicles. Vesicles were incubated at 37°C for 5 min with 30 μ M MPAG (B) or 5 μ M CDCE (C) in the presence of immunosuppressive agents cyclosporine (CsA), tacrolimus (TAC) and sirolimus (SIR). MK-571 was used as the inhibitor of MRP2. ATP-dependent transport activity was calculated by subtracting the transport activity in the presence of AMP from that in the presence of ATP. Values represent mean $\pm$ SE for three replicates.

Table 1

Apparent permeability values [P_{app} (unidirectional translocation of analyte)] and efflux ratios ($P_{appBL>AP}/P_{appAP>BL}$) in MDCKII/MRP2 cells for mycophenolic acid and metabolites.

	Apparent permeability (P_{app} , cm/second) $\times 10^6$		Efflux ratio
	BL to AP	AP to BL	
Mycophenolic acid (mg/L)			
1.0	13.83	9.83	1.40
10.0	1.83	1.05	1.74
Mycophenolic acid glucuronide (mg/L)			
5.0	34.50	6.00	5.75
50.0	5.16	0.83	6.21
Acyl-Mycophenolic acid glucuronide (mg/L)			
1.0	61.83	8.00	7.73
5.0	14.60	1.84	7.93

AP, apical; BL, basolateral; Efflux ratio, $P_{appBL>AP}/P_{appAP>BL}$; incubation period was 180 minutes; MPA and metabolite concentrations were quantified by high-performance liquid chromatography with ultraviolet detection.