The pharmacokinetic interaction between mycophenolic acid and cyclosporine revisited: a commentary on "Mycophenolic acid glucuronide is transported by multidrug resistance-associated protein 2 and this transport is not inhibited by cyclosporine, tacrolimus or sirolimus".

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The pharmacokinetic interaction between mycophenolic acid and cyclosporine revisited: a commentary on "*Mycophenolic acid glucuronide is transported by multidrug resistance-associated protein 2 and this transport is not inhibited by cyclosporine, tacrolimus or sirolimus*".

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In the past 10 years, mycophenolic acid (MPA) has become a cornerstone agent in immunosuppressive therapy. Since the earliest pharmacokinetic studies, the crucial role of its glucuronidation has been highlighted (Bullingham *et al.*, 1998). MPA is extensively metabolized to its inactive hydroxy-β-glucuronide (MPA-phenyl-glucuronide; MPAG) and the metabolite contributes to mycophenolic acid enterohepatic circulation after deglucuronidation in the gut. This feature is important since it accounts for 10% to 61% of total MPA exposure (Bullingham *et al.*, 1998). It is usually reflected as a second increase in the MPA time concentration curve, occurring 6 to 12 hours after oral dosing. Clinically relevant differences in drug exposure depending on the calcineurin inhibitor (cyclosporine A or tacrolimus) given with mycophenolate mofetil (MMF) were described and attributed to an interaction with MPA enterohepatic cycling. The combination of MMF with tacrolimus as compared to cyclosporine in renal transplant recipients was found to give rise to higher residual concentrations and Area Under the Concentration curves (AUC) of MPA (Hubner *et al.*, 1999, Zucker *et al.*, 1997). Additional studies including a "control" group of patients receiving MMF without a calcineurin inhibitor suggested that cyclosporine but not tacrolimus is responsible for the interaction (Pou *et al.*, 2001, Smak Gregoor *et al.*, 1999). This was further demonstrated in Lewis rats: animals receiving MPA with cyclosporine showed a lower exposure to MPA, higher levels of MPAG, and less second MPA peak attributable to enterohepatic drug recirculation as compared to rats receiving MPA alone or with tacrolimus (van Gelder *et al.*, 2001).

The hypothesis most often put forward is that cyclosporine decreases the biliary excretion of MPAG and thus the extent of enterohepatic recirculation. Evidence that this effect might result from the inhibition of the Multidrug Resistance-associated Protein 2 (MRP2) came from studies conducted in specific strands of rats deficient for the canalicular transporter (Hesselink *et al.*, 2005, Kobayashi *et al.*, 2004, Westley *et al.*, 2006) but this has never been

verified using a human model until now. In addition, cyclosporine was also found to result in lower plasma MPA/MPAG AUC ratio in mrp2-deficient rats, which suggests that mrp2-independent mechanisms may be involved in this interaction (Hesselink *et al.*, 2005).

In their article entitled "*Mycophenolic acid glucuronide is transported by multidrug resistance-associated protein 2 and this transport is not inhibited by cyclosporine, tacrolimus or sirolimus*", Patel et al. are the first group to study the interaction of cyclosporine on MPA enterohepatic cycling using in vitro models expressing human (and not rodent) MRP2 (Patel *et al.*, 2012). They confirmed that MPAG is a substrate for the human transporter and showed for the first time that cyclosporine does not inhibit the activity of the transporter expressed in SF9 membrane vesicles. Based on results obtained using Madin-Darby Canine Kidney (MDCK) Cells transfected with MRP2, the authors suggest that cyclosporine would inhibit the uptake of MPAG at the basolateral membrane rather than its efflux by MRP2.

Using HEK293-transfected cells, we recently found that MPAG is a substrate for the Organic Anion Transporting Polypeptide (OATP) 1B3 and 1B1 (Picard *et al.*, 2010), two uptake transporters specifically expressed at the basolateral membrane of hepatocytes. These transporters are thus likely to be involved in the interaction between MPA and cyclosporine. Consistent with this hypothesis, cyclosporine was reported as a potent inhibitor of both OATP1B1 and OATP1B3 in several in vitro studies (Shitara *et al.*, 2003, Shitara *et al.*, 2012, Treiber *et al.*, 2007). It was also demonstrated that tacrolimus has less inhibitory effect on these transporters than cyclosporine. The concentration of tacrolimus to produce 50% inhibition (IC_{50}) of OATP activities is higher than 1 µM, while that of cyclosporine is within 0.1-1µM (Fehrenbach *et al.*, 2003, Shitara *et al.*, 2012). Specifically regarding the OATP1B3-mediated uptake of MPAG, we found that 1µM cyclosporine resulted in almost complete inhibition of uptake in transfected cells (94 ± 11 %) whereas the same concentration of tacrolimus had almost no effect (9 ± 16 % inhibition) (Picard *et al.*, 2011). Interestingly

enough, Shitara *et al.* (2012) showed that cyclosporine but not tacrolimus has a long-lasting inhibitory effect on OATP1B1 and OATP1B3 activities using transfected cells as well as using human hepatocytes (Shitara *et al.*, 2012). These different inhibitory capabilities, as well as the fact that cyclosporine therapeutic blood levels are substantially higher than those of tacrolimus would explain that cyclosporine but not tacrolimus resulted in increase exposure to MPA in clinical settings.

The different effect of tacrolimus and cyclosporine on OATPs is also consistent with other reports on drug-drug interactions. This interaction between cyclosporine and cerivastatin has been essentially ascribed to the inhibition of active hepatic uptake by OATPs (Shitara *et al.*, 2003). No interaction was described between this drug and tacrolimus (Lemahieu *et al.*, 2005). The significant interaction between the endothelin receptor antagonist bosentan and cyclosporine was also entirely attributed to the inhibition of OATP transporters by cyclosporine (Treiber *et al.*, 2007) and no interaction was reported in the case of tacrolimus.

Based on these data and the evidence provided by Patel et al., we suggest that drug-drug interaction between cyclosporine and MPA, initially attributed to MRP2 inhibition is due to the inhibition of MPAG-mediated uptake in hepatocytes by OATPs.

This leads to a reinterpretation of MPA hepatic disposition: after production in hepatocytes, MPAG is partly excreted into bile but also presumably transported into the blood sinusoids by active transporters to be further eliminated by the kidneys (the major elimination pathway of the metabolite). Circulating MPAG can secondarily be taken back up into hepatocytes by OATPs to be eliminated through the bile (Figure 1). Cyclosporine interacts with this transport by inhibiting OATPs in a greater extent than tacrolimus does.

On the other hand, renal elimination of MPAG involves an active uptake of the metabolite by the organic anion transporter 3 (OAT3; SLC22A8), located at the basolateral side of proximal tubular cells (Uwai *et al.*, 2007, Wolff *et al.*, 2007), followed by an apically-directed efflux in

urine, presumably by MRP2 which was found to be expressed at this level (Schaub *et al.*, 1999). This process has not been reported to be inhibited by calcineurin inhibitors.

OATP transporters should be considered for the future evaluation of drug-drug interaction with MPA. It is likely that these transporters play a significant role in the entero-hepatic cycling of other drugs.

Declaration of interest

The author has no conflict of interest to report.

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Figure captions

Figure 1: proposed pathways for MPA hepatic disposition and enterohepatic cycling, and mechanism for its interaction with cyclosporine. The figure is based on evidence provided by the literature (Bullingham *et al.*, 1998, Patel *et al.*, 2012, Picard *et al.*, 2011, Picard *et al.*, 2010, Uwai *et al.*, 2007, Wolff *et al.*, 2007)

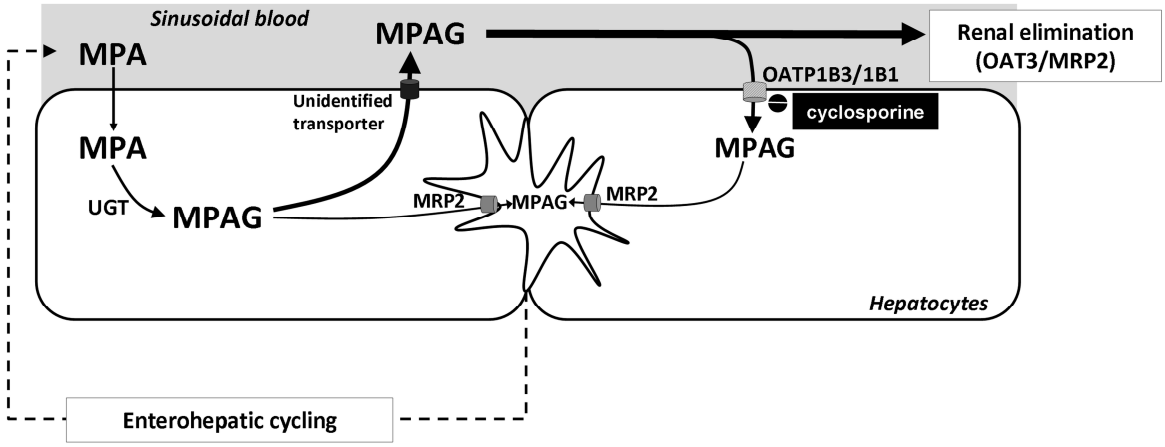
Sinusoidal blood
MPA
MPAG
Renal elimination
(OAT3/MRP2)
OATP1B3/1B1
cyclosporine
Unidentified
transporter
MPA
MPAG
UGT
MPAG
MRP2
MPAG
MRP2
Hepatocytes
Enterohepatic cycling

Figure 1