**Manuscript**:

**Oral antibiotics lower mycophenolate mofetil drug exposure by interfering with the enterohepatic recirculation: a case series**

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**Abstract**

Mycophenolate mofetil has an important role as immunosuppressive agent in solid organ transplant recipients. Exposure to the active mycophenolic acid (MPA) can be monitored using therapeutic drug monitoring. We present three cases in which MPA exposure severely decreased after oral antibiotic co-administration. By diminishing gut bacteria β-glucuronidase activity, oral antibiotics seem to prevent deglucuronidation of the inactive MPA-7-O-glucuronide metabolite to MPA and thereby prevent its enterohepatic recirculation. This pharmacokinetic interaction could result in rejection, which makes it clinically relevant in solid organ transplant recipients, especially when therapeutic drug monitoring frequency is low. Routine screening for this interaction, preferably supported by clinical decision support systems, is advised.

**Introduction**

Mycophenolate mofetil (MMF) is the backbone of immunosuppression in solid organ transplantation patients to reduce the risk of rejection.1-3 MMF interferes with de novo synthesis of purine nucleotides in B- and T-lymphocytes by reversibly inhibiting inosine monophosphate dehydrogenase. This results in decreased lymphocyte proliferation and decreased antibody production.4,5

Upon ingestion, the pro-drug MMF is rapidly hydrolyzed to the active metabolite mycophenolic acid (MPA; Figure 1).6 MPA is metabolized by uridine diphosphate-glucuronosyltransferase (UGT) isoenzymes into the inactive MPA-7-O-glucuronide (MPAG).7 MPAG is excreted via the bile to the intestines and deglucuronidated into MPA again by β-glucuronidase enzymes present in intestinal bacteria. MPA is then reabsorbed into the circulation.6 This enterohepatic recirculation of MPA comprises up to 60% of the exposure.6

MPA and its metabolites exhibit large interindividual pharmacokinetic variability. A relationship between MPA concentrations and allograft rejection has been documented, which makes therapeutic drug monitoring (TDM) of MPA an important tool to prevent inadequate drug concentrations resulting in organ rejection or MPA toxicity.8-10 The most adequate measurement of MPA exposure is a 12-hour area under the concentration-time curve (AUC[0-12h], reference: 30 – 60 mg\*h/L). The MPA Cmax is measured 0-6 hours after a single dose of MMF orally. Asecond peak after 6 hours represents re-absorption of MPA through enterohepatic recirculation.6 This method is sometimes used in clinical setting to address MPA exposure, however steady state MPA trough levels are more frequently assessed as a surrogate parameter for exposure.

Emerging evidence suggests disturbance of the MPA exposure during concomitant use of other drugs, e.g. antibiotics.11,12 We discuss three patients with decreased MPA concentrations during concomitant oral antibiotics.

**Case #1**

A 53-year-old male patient received a liver transplant because of end stage liver disease caused by primary sclerosing cholangitis. He started on tacrolimus, MMF and prednisolone orally. Because of adequate tacrolimus concentrations, MMF was discontinued. For an intra-abdominal infection, intravenous vancomycin and ciprofloxacin were administered. Because of neurological side effects of tacrolimus, the tacrolimus dose was reduced and MMF was reintroduced. The first plasma concentration of MPA (day 4; Figure 2A) was 1.48 mg/L (reference 1-3 mg/L). However, on day 8, the MPA concentration was very low and remained low (0.19-0.25 mg/L) after increasing the MMF dose to 1000mg t.i.d. Concomitant albumin levels were normal (36-42 g/L, reference 35-50 g/L). As on day 6 ciprofloxacin was switched to oral, an oral ciprofloxacin-induced disturbance of MPA concentrations was suspected. On day 20, all antibiotics were discontinued and on day 22 the MPA concentration was above therapeutic range: 3.47 mg/L. The MMF dose was then reduced to 1000mg b.i.d.

**Case #2**

A 13-year-old boy was admitted to the hospital for an elective living related kidney transplantation because of congenital uropathy. He received immunosuppression with tacrolimus, MMF and prednisolone orally. When the patient developed diarrhea, proven due to intestinal *Clostridium* *difficile* by fecal PCR, oral vancomycin was started for 10 days. Soon thereafter, MPA plasma concentrations decreased to undetectable levels (Figure 2B). After increasing the MMF dose to 1000mg b.i.d. and stopping vancomycin, MPA concentrations increased again to above therapeutic range. Unfortunately, due to a persistent *Clostridium* infection, the patient received prolonged vancomycin therapy and short courses of ciprofloxacin and amoxicillin/clavulanic acid. On several occasions, subtherapeutic MPA concentrations and AUC[0-12h]s were measured for which the MMF dose was adjusted to doses ranging from 1000 to 2500 mg per day, taking into account vancomycin dose changes. Upon lowering the dose and eventually stopping vancomycin, MPA concentrations increased quickly to (above) therapeutic range and MMF was reduced to 500mg b.i.d.

**Case #3**

A 13-year-old girl visited the emergency room with a fever, abdominal pain and a suspected urinary tract infection, 1 year and 9 months since her second renal transplant. She was on immunosuppression with tacrolimus and MMF with adequate exposure (AUC[0-12h] 51 mg\*h/L). While awaiting urine cultures, amoxicillin/clavulanic acid 500/125 mg t.i.d. was administered for 10 days (Figure 2C). On day 4, she reported at the outpatient clinic to feel much better although now having diarrhea. The MPA concentration was undetectable. The diarrhea was a suspected side effect of amoxicillin/clavulanic acid, but not likely to be solely responsible for the undetectable MPA plasma concentration. Seven days after completing the antibiotic course, the MPA concentration was in range: 2.52 mg/L (reference >1,9 mg/L).

**Discussion and conclusion**

We describe three patients with a significant reduction in MPA exposure after starting oral antibiotics, which increased again after lowering the dose or discontinuation of the antibiotics. These patients were all medication adherent – both anamnestically and proven by administration registration and/or TDM. None of the patients was co-treated with ciclosporin, which is known to inhibit the MPA enterohepatic recirculation, or any other medication that interacts with MMF or its metabolites.13

Our findings are in line with the few available small cohorts and case series. It has been shown that many oral antibiotics can cause this interaction with MMF, including rifampicine, norfloxacine/metronidazole, selective bowel decontamination (mycostatin/tobramycin/cefuroxime), ciprofloxacin and amoxicillin/clavulanic acid.11,12,14-18 Furthermore, the summary of product characteristics of MMF-originator CellCept® specifically mentions ciprofloxacin, amoxicillin/clavulanic acid, norfloxacin/metronidazole and trimethoprim/sulfamethoxazole for this interaction.13 To the best of our knowledge, we are the first to report a similar effect for oral vancomycin.

The exact mechanism underlying the interaction is still unclear. As the AUC[6-12h] and MPA trough concentration are affected predominantly, without a significant effect on AUC[0-6h], Cmax or tmax, the suggested mechanism of the interaction is interference with the enterohepatic recirculation.16 Not only a rapid decline of MPA trough levels after start of antibiotic treatment, but also rapid recovery after cessation of antibiotics have been observed. This suggests that the deglucoronidating activity of the gut flora and accordingly enterohepatic circulation can be reconstituted.17 As shown in an *in vitro* experiment, the reduction of MPA exposure might not solely depend on eradication of β-glucuronidase producing bacteria, but also on direct non-competitive inhibition of intestinal β-glucuronidase activity.19 This is also illustrated by case 1, in which MPA trough levels recovered very quickly after withdrawal of ciprofloxacin. The enterohepatic circulation interfering effect seems antibiotic-specific rather than a group effect, as inhibition of *in vitro* β-glucuronidase was observed for ciprofloxacin and enoxacin but not for levofloxacin and ofloxacin.19

Recently, more evidence is appearing regarding the influence of immunosuppressants on the gut microbiome. Tacrolimus and prednisolone are associated with pro-inflammatory dysbiosis, and alterations in the intestinal barrier and MMF is associated with pro-inflammatory dysbiosis and increased endotoxemia.20 In mice it is shown that MMF was responsible for an increase in *Clostridia* and *Bacteroides* spp. β-Glucuronidase is expressed by *Bacteroides* and as a consequence MMF stimulates the activity of gut β-glucuronidase in the cecum and the colon.21 Furthermore, in these mice it was shown that addition of vancomycin was responsible for a decrease in *Bacteroides*, B-glucuronidase activity, and free MPA in mice stool.21 *Bacteroides* are a genus of gram-negative bacteria. Therefore, antibiotics against gram-negative bacteria might be suspect for having a significant impact on the MPA blood levels in transplant recipients.

Because the enterohepatic recirculation may account for up to 60% of the MPA AUC[0-12h] and bacterial infections are common in patients using immunosuppressants such as MMF, interference with the enterohepatic recirculation by antibiotics may have a significant impact on MPA exposure and result in potentially ineffective immunosuppression.6 In a clinical setting, TDM of MPA is performed regularly. However, outpatient prescribers less familiar with transplant patients, may start antibiotics for various indications. However, this interaction is not regularly monitored and many physicians and (community) pharmacists’ are not aware of this effect of oral antibiotics on MMF. Furthermore, most of these interactions are not included in clinical decision support systems, which makes routine identification and management of these interactions difficult.

Without digital monitoring for the MMF-antibiotics interaction, medication reconciliation is essential for prescribers to be informed about the current (antibiotic) drug use by their patients. Interference can be detected using TDM. One should bear in mind that the effect on the MPA plasma concentrations may reduce again with continued antibiotic use and usually diminishes within days after antibiotic discontinuation.13 Finally, it is important to take into account that the enterohepatic recirculation predominantly influences the AUC[6-12h]. Therefore, trough concentrations may not adequately represent changes in overall MPA exposure. Close monitoring of the MPA exposure (AUC[0-12h]) and graft function during and shortly after antibiotic use is necessary.

Although more prospective research is needed into which antibiotics are involved in this interaction and through which exact mechanism, we recommend caution in transplant recipients on MMF with co-prescriptions for oral antibiotics influencing gram-negative bacteria to prevent organ rejection. Furthermore, we suggest routine screening for the combination of MMF and oral antibiotics interfering with the enterohepatic recirculation, preferably using clinical decision support systems.

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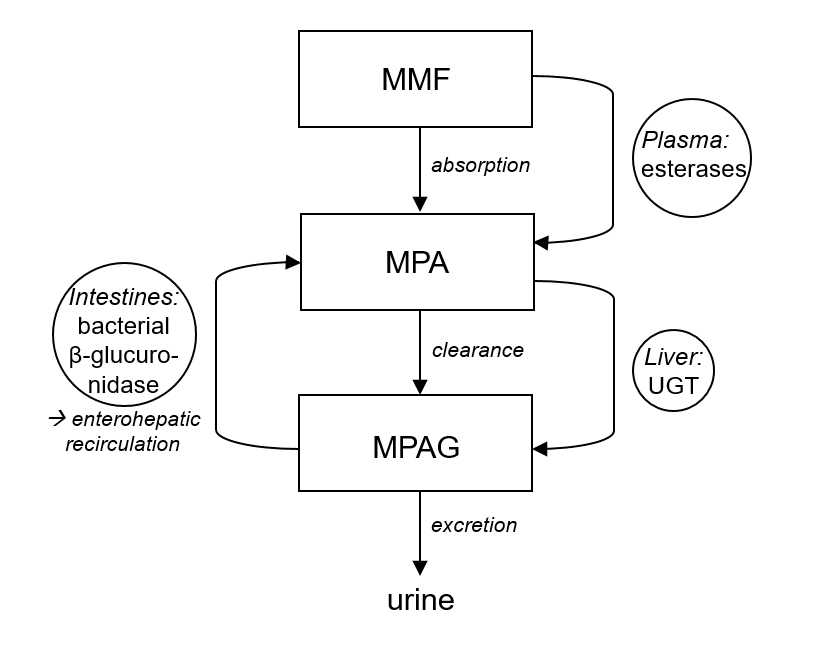
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**Figure 1. Pharmacokinetics of mycophenolate mofetil (MMF)**

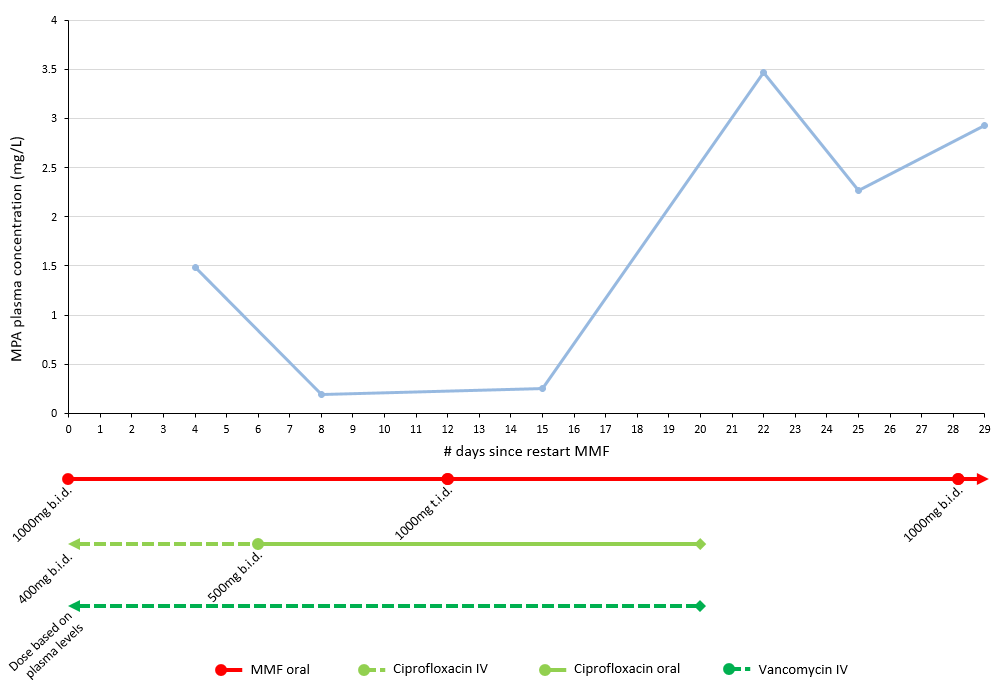


*MMF mycophenolate mofetil; MPA mycophenolic acid; MPAG MPA-7-O-glucuronide; UGT uridine diphosphate glucuronosyltransferases.*

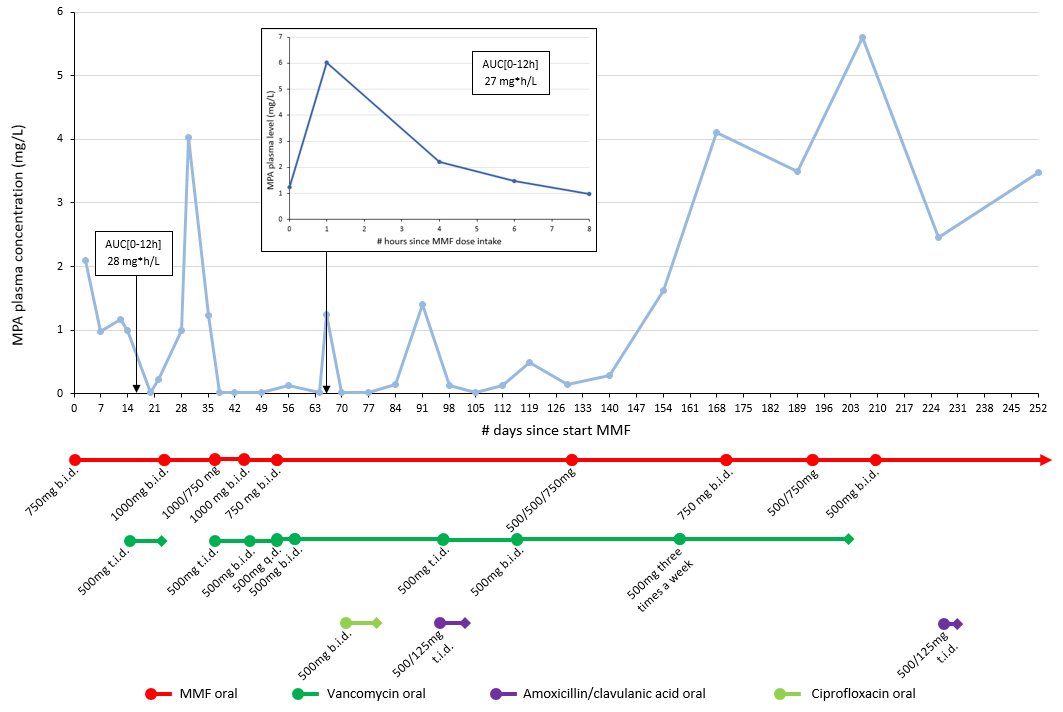
**Figure 2. Plasma MPA concentrations and co-medication over time.**

Lines connect individual measurements but do not themselves reflect measured values.A. Case 1; B. Case 2; C. Case 3.

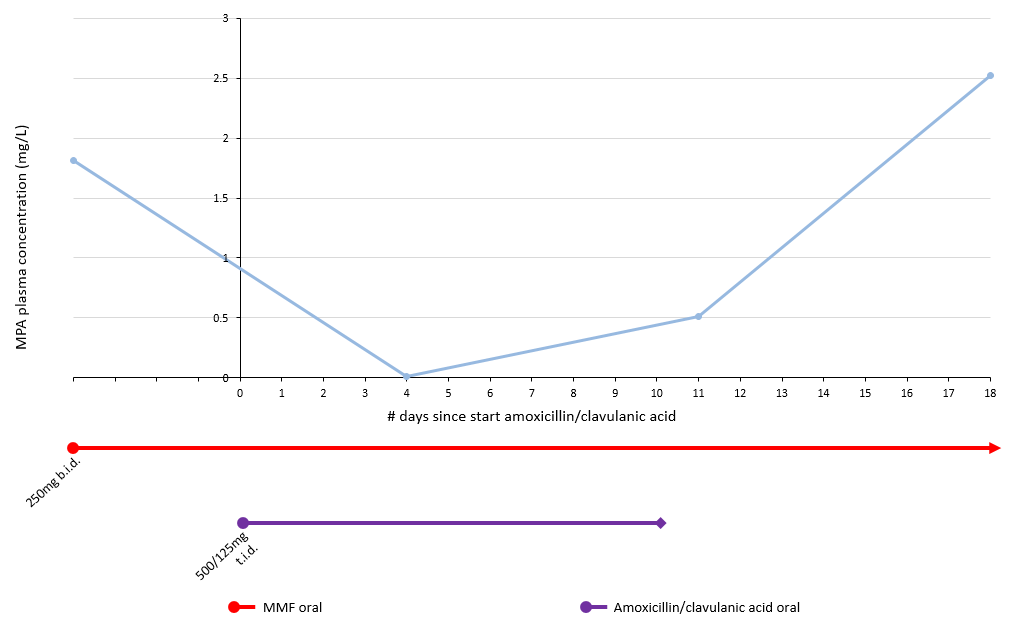
**A.**



**B.**



**C.**



*AUC[0-12h] reference 30-60 mg\*h/l. AUC area under the curve; MPA mycophenolic acid; MMF mycophenolate mofetil; b.i.d. twice daily; q.d. once daily; t.i.d. three times a day; IV intravenous.*