Renal glucuronidation and multidrug resistance protein 2-/ multidrug resistance protein 4-mediated efflux of mycophenolic acid: interaction with cyclosporine and tacrolimus

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Mycophenolic acid (MPA) is an immunosuppressant used in transplant rejection, often in combination with cyclosporine (CsA) and tacrolimus (Tac). The drug is cleared predominantly via the kidneys, and 95% of the administered dose appears in urine as 7-hydroxy mycophenolic acid glucuronide (MPAG). The current study was designed to unravel the renal excretory pathway of MPA and MPAG, and their potential drug-drug interactions. The role of multidrug resistance protein (MRP) 2 and MRP4 in MPA disposition was studied using human embryonic kidney 293 (HEK293) cells overexpressing the human transporters, and in isolated, perfused kidneys of Mrp2-deficient rats and Mrp4-deficient mice. Using these models, we identified MPA as substrate of MRP2 and MRP4, whereas its MPAG appeared to be a substrate of MRP2 only. CsA inhibited MPAG transport via MRP2 for 50% at 8 μM ($P < 0.05$), whereas Tac had no effect. This was confirmed by cell survival assays, showing a 10-fold increase in MPA cytotoxicity (50% reduction in cell survival changed from 12.2 ± 0.3 μM to 1.33 ± 0.01 μM by MPA + CsA; $P < 0.001$) and in perfused kidneys, showing a 50% reduction in MPAG excretion ($P < 0.05$). The latter effect was observed in Mrp2-deficient animals as well, supporting the importance of Mrp2 in MPAG excretion. CsA, but not Tac, inhibited MPA glucuronidation by rat kidney homogenate and human uridine 5'-diphospho-glucuronosyltransferase-glucuronosyltransferase 1A9 ($P < 0.05$ and $P < 0.01$, respectively). We conclude that MPA is a substrate of both MRP2 and MRP4, but MRP2 is the main transporter involved in renal MPAG excretion. In conclusion, CsA, but not Tac, influences MPA clearance by inhibiting renal MPA glucuronidation and MRP2-mediated MPAG secretion. (Translational Research 2014;164:46–56)

Abbreviations: CsA = cyclosporine A; FVB = friend leukemia virus B strain; HEK293 = human embryonic kidney 293; HPLC = high-performance liquid chromatography; MMF = mycophenolate mofetil; MPA = mycophenolic acid; MPAG = 7-hydroxy mycophenolic acid glucuronide; MRP = multidrug resistance protein; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OAT = organic anion transporter; Tac = tacrolimus; UDPGA = uridine 5′-diphosphoglucuronic acid; UGT = uridine diphosphoglucuronosyltransferase; WH = Wistar Hannover; WT = wild-type

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AT A GLANCE COMMENTARY

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Background

To date, renal excretion of the immunosuppressant drug, mycophenolic acid (MPA), and its glucuronide metabolite, MPAG, are not fully characterized. Here, the role of renal apical multidrug resistance associated proteins, MRP2 and MRP4 in MPA excretion, as well as in the interaction with the often coadministered immunosuppressants, cyclosporine (CsA) and tacrolimus (Tac), was studied.

Translational Significance

Using HEK293 cells over-expressing human transporters, MPA was identified as a substrate of MRP2 and MRP4 and MPAG as a substrate of MRP2 only. We confirmed our findings using isolated perfused kidneys from Mrp2-deficient rats and Mrp4-deficient mice, and demonstrated that CsA, but not Tac, inhibited renal MPA glucuronidation and MPAG renal secretion mediated via MRP2.

Mycophenolic acid (MPA) is the active moiety of the immunosuppressant prodrug mycophenolate mofetil (MMF), which is administered alone or concomitantly with other immunosuppressive drugs, such as the calcineurin inhibitors cyclosporine A (CsA) or tacrolimus (Tac), to prevent solid-organ transplant rejection.[1] Unfortunately, the use of calcineurin inhibitors is often associated with iatrogenic nephrotoxicity. MMF may improve the calcineurin-induced deterioration of renal function.[2] Furthermore, because of its immunemodulating, antifibrotic, and antiproliferative effects, MMF is prescribed to nontransplant patients to treat several immunologically mediated renal diseases.[3]

MPA is subjected to phase II metabolism in several organs, including liver, intestine, and kidney, where it is conjugated to form its main inactive metabolite 7-hydroxy-glucuronide mycophenolic acid (MPAG) via uridine diphosphoglucuronosyltransferase (UGT) enzymes.[4] In addition, some minor metabolites, including acyl-glucuronide, 7-*O*-glucoside, and acyl-glucoside are produced.[5] The acyl-glucuronide is the only metabolite that retains some *in vitro* immunosuppressive activity. MPAG, the major MPA metabolite, may reach as much as 100-fold higher concentrations than its parent drug in plasma,[6] with 82% bound to plasma albumin.[7] MPAG is secreted actively via bile into the intestine, where it is deconjugated to MPA by bacterial β-glucuronidase and reabsorbed from the colon. Enterohepatic recycling of MPAG contributes to nearly 40% of MPA exposure and results in a secondary MPA peak observed 8–12 hours after MMF administration.[8] When MMF is given in combination with CsA, the second peak of MPA is diminished,[9] and nearly double the dose of MMF is needed to achieve similar MPA levels to that when MPA is administered alone or in combination with Tac.[8] As a possible mechanism for such pharmacokinetic interaction, several studies suggested that CsA interferes with the biliary excretion of MPAG,[10,11] possibly via inhibiting MPAG active transport by hepatic multidrug resistance protein (MRP) 2.[12-14]

The kidney, however, is the main organ for MPA elimination. About 95% of the administered MMF is recovered in urine almost exclusively as MPAG, with only less than 1% as MPA.[5] Severe renal impairment in patients using MMF results in as much as a 6-fold decrease in MPAG plasma clearance.[8] In the kidney, MPA and MPAG are subjected to filtration and active tubular secretion. In addition to the liver, glucuronidation of MPA can also occur in renal proximal tubular cells, a process that could become more relevant during hepatic impairment.[4,15] MPA and MPAG interact with basolateral human renal organic anion transporter (OAT) 1 and OAT 3, which mediate their active uptake from blood into the proximal tubule cells.[16] The second step involves urinary excretion. At the renal apical membrane, MRP2/adenosine triphosphate-binding cassette transporter subfamily C2 (*ABCC2*) and MRP4/adenosine triphosphate-binding cassette transporter subfamily C4 (*ABCC4*) are involved in active renal secretion of a wide range of clinically important drugs.[17,18] However, to date, their contribution to the renal elimination of MPA and MPAG is not yet elucidated fully. Recently, Patel et al[19] showed that MPAG is a substrate of MRP2, but they could not resolve the overall renal handling of MPA. Furthermore, the effect of calcineurin inhibitors on renal MPAG excretion has not been clarified.

Here, the role of MRP2 and MRP4 in renal MPA and MPAG excretion is characterized using human embryonic kidney 293 (HEK293) cells overexpressing the human transporters. Furthermore, we investigated the renal handling of MPA at the organ level, using isolated perfused kidneys from wild-type and Mrp2-deficient rats and Mrp4 knockout mice. We identified MPA as a substrate of both transporters and MPAG as a substrate of MRP2. We also found that CsA, but not Tac, inhibits renal secretion of MPAG via combined inhibition of MRP2-mediated MPAG transport, and inhibition of renal MPA glucuronidation. In addition, we demonstrated the inhibitory effect of CsA on