

MRP4 is responsible for the efflux transport of mycophenolic acid β -D glucuronide (MPAG) from hepatocytes to the blood

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Background: Mycophenolic acid (MPA) is an immunosuppressive drug used to treat or prevent graft rejection. MPA is metabolized in the liver into mycophenolic acid β -D glucuronide (MPAG) that is present in blood at higher concentrations than MPA (10 to 50-fold). This suggests an active basolateral transport of MPAG from liver cells to the bloodstream. Among other membrane transporters, MRP4 could contribute to this efflux process, as it is known to transport a variety of glucuronide conjugates.

Method: We first developed a reproductive and robust vesicular transport assay (using overexpressing membranes from Solvo Biotechnology) to study the interaction between MRP4 and MPA (50 μ M) or MPAG (25-500 μ M). Then, we confronted the results obtained *in vitro* to *in silico* molecular docking and molecular dynamic simulations.

Results: MPAG (but not MPA) showed an ATP-dependent transport driven by MRP4 with a Michaelis-Menten constant of $233.9 \pm 32.8 \mu$ M. These observations were supported by the *in silico* calculations, rationalizing the modes of binding of MPAG in the MRP4 protein chamber. After addition of ibuprofen, cefazolin, cefotaxim and micafungin at expected therapeutic blood concentrations, the ATP-dependent transport of MPAG (25 μ M) was decreased by 84, 53, 50 and 43 % respectively.

Conclusion: MRP4 is responsible for the efflux of MPAG from the liver to the bloodstream which could explain the high concentrations observed in blood for this metabolite. MPAG may indirectly contribute to the overall immunosuppression activity of MPA when its transport is inhibited. Indeed, by inhibiting MRP4 at the basolateral side of hepatocytes, MPAG could accumulate much more in the liver and be excreted into the bile by MRP2 where it may be deconjugated back to MPA thus increasing its enterohepatic cycle

Keywords: Mycophenolic acid, Mycophenolic acid β -D glucuronide, Multidrug Resistance-Associated Protein 4, Drug-drug interactions, NSAID, anti-infectious drugs.