SVEUČILIŠTE U ZAGREBU
FARMACEUTSKO-BIOKEMIJSKI FAKULTET

ANA BORIĆ BILUŠIĆ

Učinak farmakogenetičkih varijacija UGT1A9, CYP3A4 i CYP3A5 na farmakokinetiku mikofenolne kiseline primjenjene s ciklosporinom ili takrolimusom u bolesnika s presađenim bubregom

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Effect of pharmacogenetics variants of the UGT1A9, CYP3A4 and CYP3A5 on pharmacokinetic of mycophenolic acid administered in commedication with cyclosporine or tacrolimus in renal transplant recipients

DOCTORAL DISSERTATION

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SAŽETAK

Cilj je ovoga istraživanja ispitati utjecaj polimorfizama metaboličkih enzima UGT i CYP te ABC transportera na bioraspoloživost mikofenolne kiseline (MPA) u stanju dinamičke ravnoteže primijenjene u kombinaciji s ciklosporinom (CsA) ili takrolimusom (TAC) kod bolesnika s transplantiranim bubregom. Analizirana je povezanost genske varijabilnosti metaboličkog enzima UGT1A9 te membranskog prijenosnika ABCG2 s bioraspoloživosti MPA-a. Dodatno je provedena i analiza utjecaja genske varijabilnosti metaboličkih enzima CYP3A4 i CYP3A5 s koncentracijama CsA-a i TAC-a te su procijenjeni dometi interakcije MPA-a s CsA-om i TAC-om u odnosu na ispitane varijante gena *ABCG2* u bolesnika s transplantiranim bubregom.

U istraživanje je bilo uključeno je 68 bolesnika (n=68, muškarci=35, dob 16-71) na standardnom imunosupresivnom protokolu koji uključuje MPACsA ili TAC te kortikosteroide. Uzorci krvi uzeti su tjedan dana nakon početnoga doziranja. Uzorak krvi za određivanje ostatnih koncentracija MPA, CsA i TAC-a, uzet je u 8 h ujutro prije jutarnje doze lijeka nakon čega je uslijedila standardna jutarnja doza. Tijekom 12-satnoga intervala doziranja za MPA je uzeto 6 uzoraka krvi za određivanje koncentracije MPA. MPA je analizirana HPLC metodom, a genotipizacija polimorfizama *UGT1A9 -2152C>T* i *-275 T>A, CYP3A4*22, CYP3A5*3, ABCG2 c.421C>A, ABCC2 24 C>T* i *1249 G>A, ABCB1 2677G>T/A*, *3435C>T*, *1236C>T, SLCO1B1 c.521T>C* je provedena metodom lančane reakcije polimeraze u stvarnom vremenu (qPCR). Bolesnici homozigoti divljega tipa i nositelji varijantnoga alela *ABCG2 c.421C>A* ujednačeni su metodom egzaktnoga uparivanja u kombinaciji s optimalnim potpunim uparivanjem u odnosu na demografske, biofarmaceutske i genetičke varijable te je procijenjen učinak varijantnoga alela (frequentist, Bayes) usporedbom omjera geometrijskih srednjih vrijednosti (GMR) farmakokinetičkih parametara MPA ujednačenih prema dozi u stanju dinamičke ravnoteže. Dobiveni rezultati ukazuju na značajnu povezanost varijantnoga alela *ABCG2 c.421C>A* i farmakokinetike MPA-a. Kod stabilnih bolesnika s transplantiranim bubregom koji su ujedno i nositelji varijantnoga alela *ABCG2 c.421C>A,* rezultati ukazuju na povećanu bioraspoloživost MPA ($AUC_{\tau,ss} \approx$ 40 %) u skladu s proporcionalno smanjenim klirensom ($CL_{T/F,ss} \approx$ 30 %). Dodatno je uočena značajna uloga polimorfizma *ABCG2* c.*421 C>A* u moduliranju interakcije CsA-a i TAC-a s MPA-a. Rezultati upućuju na znatno izraženiji učinak varijantnoga alela *ABCG2 c.421C>A* u skupini bolesnika na terapiji TAC-om kod kojih je zabilježena dva puta veća bioraspoloživost MPA, manji klirens i manja brzina apsorpcije u odnosu na skupinu bolesnika na terapiji CsA-om. Rezultati nisu pokazali utjecaj ispitivanih

polimorfizama metaboličkog enzima UGT1A9 na koncentracije MPA-a. Također nije zabilježen ni značajan utjecaj polimorfizama enzima CYP3A4 i CYP3A5 na koncentracije CsA-a i TAC-a, moguće zbog malog broja bolesnika nositelja varijantnih alela.

SUMMARY

Introduction

Mycophenolic acid (MPA) is a standard component of immunosuppresant protocols in organ transplantation administred in concomitant therapy with calcineurin inhibitors cyclosporine (CsA) and tacrolimus (TAC). MPA is selective reversible inhibitor of inosine monophosphate dehydrogenase, potently suppresses T and B lymphocyte proliferation. Because of low MPA oral bioavailability, on the market is available in two forms, prodrug formulation an ester of mycophenolate mofetil (MMF) and enteric coated formulation of mycophenolate sodium (EC-MPS). MPA pharmacokinetic is very complex and variable hence it is subject to therapeutic drug monitoring (TDM). Different factors interfere with exposure to MPA, including age, body mass index, renal function, changes of gut microbioma, reduced albumin levels, interactions with food, drug-drug interactions mainly with CsA/TAC, MPA formulations and genetics. MPA (around 90% of bioavailable fraction) is extensively metabolised in liver (to some extent also in the intestine and kidneys) by glucuronidation to inactive MPA-phenyl-glucuronide (MPAG) and a minor acyl-glucuronide. Enzyme UGT1A9 plays a dominant role in hepatic metabolism of MPA by minor contribution of other UGT's. The primary inactive metabolite MPAG is transported from liver cells into bile through ATP-binding cassette transporter, multidrug resistance – associate protein 2 (MRP2, encoded by gene *ABCC2*). MPAG enters gastrointestinal tract where under catalytic action of glucuronidase from intestinal flora, is hydrolyzed back to MPA (deglucuronidation) which is then recycled into blood. This process is enterohepatic recirculation pathway accounting for 10 - 61 % of total MPA exposure resulting in second increase of MPA concentration 6-12 hours after oral administration. Final elimination of MPAG and MPA (negligible amounts) is by kidney by active secretion (possibly mediated by ABCC2) therefore reduced renal function reduces MPAG/MPA elimination and increases total circulating MPA. The most valuable pharmacokinetic parameter for MPA exposure estimation is area under concentration-time curve (AUC) hence is recommended that for optimal effectiveness in renal transplantation, MPA AUC should be within a narrow range between 30 - 60 mg/L/h. Available studies reported CsA effect on MPA AUC, results showed lower MPA AUC and trough concentrations in patients receiving CsA compared to those co-treated with TAC. MPA is substrate of efflux transporter multidrug resistance protein 1 (MDR-1/encoded by *ABCB1*), MRP2 and influx organic anion transporter polypeptides, OATP1B1 and OATP1B3 – these transporter proteins move MPAG in and out of hepatocytes and renal tubular cells. One more efflux transporter seems to be included in MPA exposure – breast

cancer resistance protein (BCRP, *encoded by ABCG2*) important for membrane transport of numerous drug in intestine, liver and kidney. ABCG2 is expressed on enterocytes apical membrane (intestinal absorption regulation) and on hepatocytes canalicular membrane (transport from liver to bile). Since its primary function is control of intestinal absorption and secretion of drugs from liver to bile, lower ABCG2 activity due to polymorphisms or drug interactions (transporter inhibition), can significantly change the pharmacokinetics of administred drugs. Polymorphism *ABCG2 c.421C>A* is associated with ABCG2 reduced activity that consequently causes increased systemic exposure of substrates and occurrence of side effects.

The aim of the proposed research was to analyse the influence of metabolic enzyme UGTs, CYPs and ABCs transporter polymorphism on steady-state mycophenolic acid (MPA) exposure applied in commedication with cyclosporine (CsA) or tacrolimus (TAC) in renal transplant recipients. Study is limited by lower number of *UGTs* and *CYPs* variant carriers therefore the focus will be on statistically significant results for the *ABCG2 c.421C>A* polymorphism assocciation with PK of MPA.

Materials and Methods

Study included consecutive adult and adolescent de novo renal transplant recipients, a total of 68 patients (n=68, male=35, age 16-71) treated with mofetilmycophenolate (n=23, 2x500 do 2x1000 mg/day) or entero-cotaed mycophenolate sodium tablets (n=45, 2x720 mg/day), CsA or TAC and corticosteroids were submitted to routine therapeutic drug monitoring (TDM) of immunosuppressants after completion of initial week of treatment. All participants provided signed informed consent for genotyping of pharmacogenes. Patients on standard immunusuppressants protocol were closely monitored over 5-7 posttransplant days. On the subsequent day (steady-states of MPA, CsA and TAC achieved), after overnight fast, blood samples for quantification of MPA, CsA and TAC were taken at 8.00 h before the next morning dose. Treatments were administred and six blood samples were taken over 12 h dosing interval (at 0.5, 1, 2, 3, 8 and 12 h post-dose) for quantification of MPA. They were included in the present analysis if: 1) clinical status was considered stable during the observed period based on (i) lack of surgical complications and signs of graft dysfunction or rejection; (ii) no severe comorbidity (cardiovascular, hepatic, metabolic, infectious, gastrointestinal); (iii) low immunological risk, (iv) stably improving renal function (serum creatinine ≤ 300 μmol/L and by at least 1/3 lower than on the 1st postoperative day, with stable diuresis at around 60

mL/hour); (v) serum albumin >31 g/L; 2) were not treated with drugs that affect exposure to MPA (proton pump inhibitors, antacids, phosphate binders, oral iron, magnesium or calcium, rifampicin or any antibiotics) during the pre-study period and for the study duration.

HPLC method was used for total plasma MPA analysis, polymorphisms genotyping of *UGT1A9 (-2152C>T, -275 T>A), CYP3A4*22, CYP3A5*3, ABCG2 c.421C>A, ABCB1 2677G>T/A*, *3435C>T*, *1236C>T, SLCO1B1c.521T>C* was performed on Applied Biosystems 7500 Real-Time PCR method. Whole blood CsA and TAC were determined by a validated affinity chrome-mediated immunoassay (ACMIA, Siemens, Germany), creatinine clearance was estimated (Cockroft – Gault) based on serum creatinine quantified by an enzymatic assay on an automated analyzer (Cobas c 501; Roche, Germany) validated by isotope dilution mass spectrometry. Genotyping was performed on an Applied Biosystems 7500 Real Time PCR System, according to manufacturer's instructions (Applied Biosystems, CA, USA) using a validated TaqMan® Drug Metabolism Genotyping Assays (Life Technologies, Carlsbad, CA, USA) for the following polymorphisms: *ABCG2 c.421C > A* (rs2231142, ID C_15854163_70); *ABCC2 -24C>T* (rs717620, ID C_2814642_10) and *1249G>A* (rs2273697; ID C_22272980_20); *SLCO1B1 c.521T > C* (rs4149065, ID C_30633906_10); *UGT2B7 –161C>T* (rs7668258, ID C_27827970_40); *UGT1A9-275 T>A* (rs6714486, ID C__27843087_10) and *-2152C>T* (rs17868320, ID C__34418857_10); *ABCB1 3435C>T* (rs1045642, ID C___7586657_20) and *1236C>T* (rs1128503, ID C___7586662_10)*; CYP3A4*22* (rs35599367, ID C__59013445_10) and *CYP3A5*3* (rs776746, ID C__26201809_30). Genotyping of *ABCB1 c.2677G > T/A* (rs2032582) was performed by real-time PCR genotyping on the LightCycler® instrument (Roche Diagnostics, Mannheim, Germany). Standard MPA steady-state measures, peak exposure ($C_{max,ss}$ mg/L), area under the concentration-time curve over the dosing interval of 12 hours ($AUC_{\tau,ss}$ mg × h/L), morning and evening pre-dose concentrations (C_0, C_{12}, mg/L), apparent total body clearance ($CL_{T/F,ss}$ mL/min/kg)) were determined by the non-compartmental method (Kinetica 4.1, InnaPhase Corp., USA). We calculated also the $C_{max}/AUC_{\tau,ss}$ (1/h) ratio as an indicator of the absorption rate. The analysis was based on dose-normalized concentrations (per 1000 mg) accounting for the fact that 1000 mg of MMF corresponded to 739 mg of MPA and 1000 mg of EC-MPS corresponded to 936 mg of MPA.

ABCG2 c.421C>A wild-type homozygotes versus variants were matched by full optimal combined with exact matching method with respect to demographic, biopharmaceutic and genetic variables. Variant allele *ABCG2 c.421C>A* effect was evaluated (frequentist, Bayes) by

comparing dose-adjusted steady-state MPA pharmacokinetics estimates of geometric mean ratios (GMR).

Results

Of the 68 included patients, 12 (17.7%) were *ABCG2 c.421C>A* variant allele carriers and 56 were wt subjects. Variants in *CYP3A4* and *CYP3A5* were rare. The *UGT1A9 -275T>A* and *UGT1A9 -2152C>T* SNPs were in complete LD. Consequently, patients were considered as having a wt or a variant diplotype. The three *ABCB1* SNPs were in a strong LD (pairwise D'=0.85-0.95, r^2=0.615-0.687). Therefore, patients were categorized in respect to the number of variant alleles: (i) all three genotypes are wt, or one is heterozygous (none or one variant allele); (ii) two to three variant alleles (any two or all three loci are heterozygous; or one variant homozygous and one heterozygous locus); (iii) four to six variant alleles. Variant carriers prevailed regarding *UGT2B7 -161 C>T* SNP, while wt homozygotes prevailed regarding *SLC01B1 521T>C* and *ABCC2 -24C>T* and *ABCC2 1249G>A* SNPs. In respect to these SNPs, patients were categorized as variant carriers or as wt homozygotes.

Raw data (12 variant versus 56 wild-type patients) indicated around 40% higer total MPA exposure (frequentist GMR=1.45, 95% CI 1.10-1.92; Bayes=1.38, 95% CrI 1.07-1.81) and around 30 % lower body clearance (frequentist GMR=0.66, 95% CI 0.48-0.90; Bayes=0.71, 95% CrI 0.53-0.95) in *ABCG2 c.421C>A* variant allele carriers than in wild-type controls. The estimates were similar in matched data (11 variant verss 43 wild-type patients) and report around 41% (frequentist) and 39% (Bayes) higer MPA exposure (frequentist GMR=1.41, 95% CI 1.11-1.79; Bayes GMR=1.39, 95 % CrI 1.05-1.81) associated with proportionally lower body clearance (27% frequentist; 29% Bayes). The effect was seen in TAC co-treated patients (adjusted GMRs for exposure GMRs 1.96 (1.35-2.84) and 1.95 (1.38-2.87); for clearance 0.47 (0.32-0.68) and 0.42 (0.28-0.60)) but not in CsA co-treated patients (all GMRs around 1.0). The overall effect of variant alleles *UGT1A9 (-2152C>T, -275 T>A), CYP3A4*22, CYP3A5*3* was not demonstrated.

Discussion

Present data strongly suggest that the variant *ABCG2 c.421C>A* (rs2231142) allele increases $AUC_{\tau,ss}$ of MPA in stable renal transplant patients (by around 40%, with a high probability that the effect is > 20%) in agreement with proportionally reduced $CL_{T/F,ss}$. The effect is-considerably more pronounced (2-fold increase in exposure) - only when CNI is TAC and is weak or lacking in CsA co-treated patients. The estimates are consistent based on raw data

(patients free of relevant interfering comorbidities and co-medication) and in matched/adjusted analysis, where a number of further potential confounders, “classical” and pharmacogenetic, were controlled for. The present sensitivity analysis suggests: even if it existed, and even if really marked, such a (hypothetical) cumulative confounding effect would not completely explain away the observed effect since GMR for the variant *ABCG2 c421C>A* allele vs. wild type would still be 1.20. It is justified to state that present data reasonably validly document an effect of the *ABCG2 c.421C>A* variant allele on steady-state exposure to MPA in renal transplant patients. Discrepancy between the present results and earlier studies not detecting associations between exposure to MPA and *ABCG2 c.421C>A* SNP might, at least in part, be due to methodological differences. Our patients were co-treated with CsA or TAC (and matched for CNI and CNI troughs). Neither CsA nor tacrolimus are ABCG2 substrates, but both are ABCG2 inhibitors, and their inhibitory effect might differ, particularly under *c.421* SNP (with reduced transporter numbers). Present analysis reasonably supports a conclusion that the observed difference in $AUC_{\tau,ss}$ between the *ABCG2 c.421C>A* variant and wt subjects is attributable to the fact of variant allele carriage. Present study is limited by a modestly sized single-center sample, the fact that MPAG was not measured (as not a part of routine TDM), and connected to that, by no insight into possible mechanisms of the observed effect. Nevertheless, present analysis reasonably supports a conclusion that the observed difference in $AUC_{\tau,ss}$ between the *ABCG2 c.421C>A* variant and wt subjects is attributable to the fact of variant allele carriage.

Conclusions

Loss-of-function polymorphism *ABCG2 c.421C>A* increases steady-state MPA exposure in stable renal transplant recipients on TAC therapy. Interaction between *ABCG2 c.421A* allele and MPA exposure was observed, which seems to depend on the calcineurin inhibitor type, since the MPA exposure was twice higher in patients treated with TAC not with CsA. Study failed to demonstrate overall association of polymorphism *UGT1A9 (-2152C>T, -275 T>A), CYP3A4*22, CYP3A5*3* with MPA exposure, probably due to small sample size of variant transplant patients. In conclusion, present data strongly suggest that the variant *ABCG2 c.421C>A* allele increases steady-state exposure to MPA in stable renal transplant patients.

Key words: mycophenolic acid, cyclosporine, tacrolimus, transplant patients, ABCG2 polymorphism