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Original Article

POPULATION PHARMACOKINETICS OF 2-OXO-CLOPIDOGREL IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Abstract. *The aim of the study was to develop a population pharmacokinetic (PK) model for clearance of 2-oxo-clopidogrel in patients with acute coronary syndrome (ACS). Population pharmacokinetic analysis was performed by using 72 plasma concentrations from the same number of patients (mean age of 60.82±10.76 years; total body weight (TBW) of 73.63±9.67 kg) with ACS using non-linear mixed-effect modeling (NONMEM). Validation of the final PPK model was carried out through the bootstrap analysis with 200 runs and it was used to estimate the predictive performance of the pharmacokinetic model. The typical mean value for 2-oxo-clopidogrel clearance (CL), estimated by the base model (without covariates), in our population was 39.2 l h−1.The value of aspartate transaminase and co-medication with digoxin were determinants of a derived population model. The final regression model for the clearance of 2-oxo-clopidogrel was the following: CL (lh^{-1}) = 1.7 + 1.31*AST + 115*DIGOXIN. The derived PK model describes the clearance of 2-oxo-clopidogrel in patients with ACS, showing that the value of aspartate transaminase and co-medication with digoxin are the most important covariate. This finding will provide the basis for future PK studies.*

Key words: *2-oxo-clopidogrel, acute coronary syndrome, population pharmacokinetics, clearance, Nonlinear mixed effects model (NONMEM).*

Introduction

Clopidogrel, a second generation thienopyridine, is P2Y12 subtype of adenosine diphosphate (ADP) receptor antagonist. To date, a large number of conducted studies have shown clinical benefit of treatment with clopidogrel in addition to aspirin as dual antiplatelet therapy for acute coronary syndrome (ACS) and/or undergoing percutaneous coronary intervention (PCI) [1–3]. It is still cornerstone antiplatelet therapy for coronary heart disease despite some advantages of new P2Y12 receptor antagonists such as prasugrel and ticagrelor [4, 5]. The higher treatment cost of novel antiplatelet drugs, as well as its higher risk of major bleeding, maintained clopidogrel as first –line therapy option and extensively prescribed drug worldwide.

Clopidogrel is a prodrug, and its biotransformation by a 2-step, cytohrome P_{450} - dependent process, is requested for conversion to its active metabolite. Introduction of one oxygen atom into clopidogrel, and its conversion to 2-oxo-clopidogrel is the first step of its hepatic metabolism with contribution of CYP1A2, CYP2B6, and CYP2C19 by 35.8, 19.4 and 44%, respectively [6]. In the next oxidation process, 2-oxo-clopidogrel is converted into the pharmacologically active metabolite R-130964 by CYP2B6 (32.9%), CYP2C9 (6.76%), CYP2C19 (20.6%), and CYP3A4 (39.8%) enzymes [7]. The genes encoding CYP3A4/5 and CYP2C19 are polymorphic and accountable in variability of clopidogrel pharmacokinetics. Based on the available information regarding a correlation between both pharmacokinetic parameters of clopidogrel and H4, active metabolite of clopidogrel, as well as the Cmax of the H4 isomer and platelet aggregation, the intestinal absorption of clopidogrel may be considered as rate-limiting process [6, 8]. Additionally, the latter observations allowed the prediction of pharmacodynamics response to clopidogrel using pharmacokinetic data on clopidogrel or 2-oxo-cxopidogrel. Furthermore, previous publications have suggested both active metabolite's instability and existance in the plasm for a short period [6, 9]. In accordance with the previously mentioned, in this study we evaluated the clinical and demographic factors that influence 2-oxo-clopidogrel clearance, supposing this is relevant for vicagrel, novel analog of clopidogrel and its further development in phase II/III studies [10].

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The focus of our study was the evaluation PK variability of 2-oxo-clopidogrel in patients with acute coronary syndrome (ACS) using the population PK approach.

Methods

Patient Data

The investigation was performed at the Clinic of Cardiology, Clinical Center Niš, Serbia during three months (from February to May 2016) after obtaining permission of the Ethics Committee of the University of Nis, Faculty of Medicine. All patients were informed about the details related to the study protocol and were included after their written consent. The including criteria were: patients of both sexes older than 18 years, with diagnosis of acute coronary syndrome (ACS) with/without ST-elevation as confirmed by the cardiologists, using the electrocardiogram and the biochemical tests. The exclusion criteria for our study were: pregnant and lactating women, presence of mental disorders and patients' refusal to participate in the study.

Our study population consisted of 72 patients treated with clopidogrel in accordance with Institutional Review Board/Human Subjects Research Committee requirements. The dual antiplatelet therapy (acetylsalicylic acid and clopidogrel) was administered in all patients. Clopidogrel loading dose was 300mg or 600 mg and it varied depending on the revascularization procedure (PCI or fibrinolytics) and patients' status. After that, clopidogrel was administered once daily, at the maintenance dose of 75mg or 150mg. Besides that, all patients also received angiotensin-converting enzyme *(*ACE*)* inhibitors and other drugs presented in Table 1. This data and other clinical and demographic data of patients (ejection fraction (ER), their concomitant diseases, total body weight (TBW), age of patients and sex), were obtained from medical records, while data of life habits were recorded in a conversation with patients.

Blood sampling and laboratory analysis

The study protocol included taking four blood samples from all patients: two for the routine laboratory tests immediately after the admission of patients to hospital, and two blood samples after three days of starting therapy with clopidogrel for the patients' genotyping and measuring of the drug concentrations, respectively.

Oxoclopidogrel concentrations were measured from the serum samples at the steady–state concentrations using the ultrahigh-performance liquid chromatography with diode array detector-mass spectrometry analysis (UHPLC-DAD-MS). The UHPLC was carried out on a Dionex Ultimate 3000 UHPLC system equipped with a DAD-detector and also connected to LCQ Fleet Ion Trap Mass Spectrometer (Thermo Fisher Scientific, USA). The process of separations was performed on a Poroshell 120 EC-C18 column (4.6×50mm, 2.7μm; Agilent technology, USA) at room temperature (25°C). The absorption was recorded on DAD-detector (with total spectral range between 200nm and 800nm), set at three detection wavelengths of 240, 220 and 300nm, simultaneously. Mass spectrometric analysis was performed using an LCQ 3D-ion trap mass spectrometer with electrospray ionization (ESI) in positive ion mode. MS-spectra were acquired by full range acquisition of m/z 300–500. For fragmentation study (MS/MS), a data dependent scan was performed by deploying the collision–induced dissociation at 25eV. The range of the linearity for oxoclopidogrel was …with the lowest the limit of detection and quantification of serum concentration of oxoclopidogrel was 0.5ng/mL and 50.0 ng/mL respectively. All the serum samples for this type of the analysis were obtained in Cmax concentrations, two hours after oral administration of morning dose of clopidogrel.

The patients' genotyping analysis was included before isolating manually the genomic DNA from the whole blood leukocytes and the following small nuclear polymorphisms (SNPs) which was determined by using the PCR (polymerase chain reaction) method: ABCB1 C34335T (rs1045642), CYP2C19*2 (rs4244285) and *17 (rs12248560), as well as CYP2C9*2 (rs1799853). The commercial mix KAPA2G Readymix (KAPA2G Ready-Mix FastHotStart; Kappa Biosystems, Boston, MA, USA), the reaction mixture with specific primers were used for SNPs detections. For detection of gene polymorphism of ***ABCB1 C3435T*** were used primers *C (5′−GGTGTCA CAGGAAGAGATC−3′),* (5′−CAGCCGGGTATAGTCA CAGGAAGATATT−3′) and the reverse *(5′−GGCCAGA GAGGCTGCCACAT−3′),* for the detection of gene polymorphism of ***CYP2C19*2*** primers were used the forward *(5′−AATTACAACCAGAGCTTGGC−3′),* the reverse *(5′−TATCACTTTCCATAAAAGCAAG−3′),* for the recorded of gene polymorphism of ***CYP2C19*17*** used the forward *(5′−GCCCTTAGCACCAAATTCTC−3′)* and the reverse *(5′−ATTTAACCCCCTAAAAAAACACG−3′)* and the end for the detection of ***CYP2C9*2*** gene polymorphism was used the forward *(5′−GTATTTTGGCCTGA AACCCATA−3′)* and the reverse *(5′−GGCCTTGGTT TTTCTCAACTC−3′).* The reaction condition consisted of the initiation at 95°C for 2 minutes followed by different number of cycles. In the case of ABCB1 no restriction enzyme was used, since the primers were allele specific. SNPs determination was performed after vertical electrophoresis in 8% polyacrylamide gel (ABCB1 C3435T and CYP2C19*2) or horizontal electrophoresis in 2% agarose gel (CYP2C19*17 and CYP2C9*2).

Pharmacokinetic analysis

For population pharmacokinetics (PPK) analysis of oxoclopidogrel and the estimates of its pharmacokinetics parameters (PK) of the target population, we applied the NONMEM software (version 7.3.0.) (Icon Development Solutions, USA) with ADVAN 1 subroutine which well describes a one-compartment model without absorption [11]. This model has given the best estimate of the main PK parameters of oxoclopidogrel, the apparent oral clearance (CL/F) and its inter-individual and residual (intra-individual) variability. The choice of appropriate subroutine was based on the literature data of clopidogrel and

oxoclopidogrel pharmacokinetics and testing of different PPK models (using the lowest value of the minimum of objective function (MOF) as the mean statistical criteria between the tested models). In this step, with the aim to form the base PPK model of investigated drug, we also analyzed various models errors for the estimation of variability, using an exponential, additive, proportional and combined model errors. The parent drug, clopidogrel was administered orally in all patients and the influence of oral bioavailability (F) was not considered in the analysis. The base PPK model of oxoclopidogrel was built using the collected data of 72 patients with ADVAN1 subroutine from the software library (without tested covariate).

Covariate of interest for the PPK analysis of oxoclopidogrel were the following: total body weight, age, sex, total daily dose of clopidogrel, ejection fraction, cholesterol and triglyceride levels, red blood cells (RBC) count, creatinine clearance (*calculated for each patient using the* Cockcroft-Gault *equation)*, aspartate transaminase (AST) and alanine transaminase (ALT), ABCB1 and CYP2C9 genotypes, CYP2C19 phenotype, concomitant disease as the presence of diabetes mellitus (DM) type 2, life habits such as smoking status of patient and co-medication with other drugs: beta-blockers, diuretics, spironolactone, amlodipine, amiodarone, digoxin, pantoprazole, sulphonylureas and statins (atorvastatine, rosuvastatine and simvastatine). The influence of 25 covariates on the pharmacokinetic disposition of oxoclopidogrel, were analyzed through the univariate regression models. This process consisted of adding only one of tested covariate in the base model (in a linear or nonlinear way), and then, its statistical significance was assessed on the reduction in the MOF value between this model and the base PPK model. The MOF is defined as -2loglikelihood and, therefore, its required reduction was at least 6.63 ($p<0.01$, df=1). As the results of this complex process, we recorded all covariate with the significant influences on the PK parameters of oxoclopidogrel, and after that, were added in the full model, simultaneously. Further, the analysis was performed in the opposite manner, removing each individual covariate from the full model and noting the differences in the MOF value. In this process of backward removal, the main statistical criteria was stronger, with increasing of the MOF values by $\geq$10.83 ($p<0.001$, df=1) [12]. Thus, the final PPK model of oxoclopidogrel consisted of only those covariate which satisfied both approach selection processed (forward addition and backward removal), suggesting their particular influence on pharmacokinetics of the investigated drug. Integral part of this analysis was a graphic inspection of data fitting between predicted, population values of concentration versus obtained, measured concentrations of oxoclopidogrel, which was obligatory conducted during all phases.

Validation of the final PPK model was carried out through the bootstrap analysis with 200 runs. This type of the internal validation was recommended for a small number of samples per patient by the Guidance for Industry Population Pharmacokinetics, FDA, for the estimation of the stability and predictive performances of the final PPK model [13]. Values of PK parameters and its variability from the bootsrap analysis were compared with the values from NONMEM analysis and if similar, that indicated a good predictive performance of the derived PPK model.

Results

The study population consisted of 72 patients of both sexes (28 female and 44 male) in order to assess the mean population value of the clearance of oxoclopidogrel, as the main metabolite of clopidogrel. The range of patients age was 37 to 85 years, including middle-age to elderly patients, with the average value of

Table 1 Respondent's characteristics (demographic, biochemical, genetic and clinical data)

Characteristics of population	Investigated set (mean values ± SD*)	Range of investigated set
Number of patients	72	
Number of observations	72	
Gender (male/female)	44/28	
TBW (kg)	73.63 ± 9.67	55–106
Age (years)	60.82 ± 10.76	37–85
Clopidogrel daily dose (mg/day)	84.38 ± 24.98	75–150
Oxoclopidogrel plasma concentration (mg/l)	0.29. ± 0.39	0.0008–1.7309
ABCB1 genotype:	72	
- CC	- 19	
- CT	- 34	
- TT	- 19	
CYP2C19 phenotype:	72	
- PM	- 4	
- IM	- 19	
- EM	- 25	
- URM	- 24	
CYP2C9 genotype:	72	
-CC	- 56	
-CT	- 15	
-TT	- 1	
Ejection fractions (%)	51.32 ± 12.27	28–79
Cholesterol levels (mmol/l)	5.52 ± 1.35	2.95–10.87
Triglyceride levels (mmol/l)	2.19 ± 3.85	0.14–33
RBC counts (10^{12}/l)	4.66 ± 0.68	3. 7–6.12
AST (U/l)	117.21 ±169.81	10.2–930
ALT (U/l)	34.20 ± 26.57	1.27–146.7
Creatinine clearance (l h^{-1})	4.38 ± 1.47	1.28–7.23
Diabetes mellitus	19	
Smokers	24	
Co-medications with:		
- Pantoprazole	- 58	
- Beta blockers	- 49	
- Diuretics	- 21	
- Spironolactone	-14	
- Amlodipine	-12	
- Amiodarone	- 8	
- Digoxin	- 8	
- Sulphonylureas	- 4	
- Statins:	- 69	
- Atorvastatine	- 60	
- Rosuvastatine	- 2	
- Simvastatine	- 7	

*SD- standard deviation

body weight of 74kg. Clopidogrel tablets were routinely administered orally, once daily at the dose of 75mg (n=63, 87.5%) or 150mg (n=9, 12.5%). The presence of DM type 2 was showed as the most common concomitant disease (n=19, 26.39%), while almost a third of the population were active smokers (n=24, 33.33%). In terms of co-medications with other drugs, three groups were administered most frequently: statins (95.83%) as the drugs that reduce cholesterol levels (atorvastatine (83.33%), rosuvastatine (2.78%) and simvastatine (9.72%)), the gastroprotective therapy with the proton pump inhibitors (PPIs) (pantoprasole (80.56%)) and beta blockers (BBs) (68.06%). The other co-administered drugs were: diuretics (29.17%), spironolactone (19.44%), amlodipine (16.67%), amiodarone (11.11%), digoxin (11.11%) and sulfonylurea derivatives (5.56%). The average serum concentration of oxoclopidogrel was 0.29±0.39 followed with a wide range from 0.0008 to 1.730mg per liter in the population. Overall, demographic data, biochemical, genetic and clinical data of the respondents are presented in Table 1.

The mean population value of oxoclopidogrel clearance without examining the effects of different covariate was 39.2 liters per hour in the base model. This analysis was carried out using one compartmental model with no absorption from NONMEM software, since the active metabolite was the objective of our investigation. Further, the process of forward selection was conducted in order to assess the influence of each individual factor on pharmacokinetics disposition of the drug. The values of MOF, evaluated by univariate models which examined the effects of separate factors on oxoclopidogrel clearance, are shown

Table 2 Values of MOF for the base model and univariate regression models of examined covariates with their statistical significance in the process of building of the full PPK model

Clearance models	Minimum of objective function	p-value **
BASE MODEL		
CL= θ_1 *EXP(ETA(1))	1047.867	
UNIVARIATE REGRESSION MODELS		
CL= θ_1*EXP(ETA(1)) + θ_2 *AGE	1030.711	**<0.01**
CL= θ_1*EXP(ETA(1)) + θ_3 * TBW	1046.725	>0.01
CL= θ_1*EXP(ETA(1)) + θ_4* SEX	1047.867	>0.01
CL= θ_1*EXP(ETA(1)) + θ_5* DD	1047.108	>0.01
CL= θ_1*EXP(ETA(1)) + θ_6* ABCB1	1046.007	>0.01
CL= θ_1*EXP(ETA(1)) + θ_7* CYP2C19 genotype	1046.103	>0.01
CL= θ_1*EXP(ETA(1)) + θ_8* CYP2C9 genotype	1046.477	>0.01
CL= θ_1*EXP(ETA(1)) + θ_9* EF	1047.001	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{10}* CHOL	1031.930	**<0.01**
CL= θ_1*EXP(ETA(1)) + θ_{11}* TGL	1047.449	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{12}* RBC	1047.867	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{13}* AST	990.892	**<0.01**
CL= θ_1*EXP(ETA(1)) + θ_{14}* ALT	1047.866	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{15}* CLcr	1047.138	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{16}* DM	1047.867	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{17}* TOB	1047.524	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{18}* PANT	1047.424	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{19}* BB	1047.867	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{20}* DIU	1047.867	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{21}* SPI	1047.860	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{22}* AML	1047.867	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{23}* AMI	1040.078	**<0.01**
CL= θ_1*EXP(ETA(1)) + θ_{24}* STAT	1047.867	>0.01
CL= θ_1*EXP(ETA(1)) + θ_{25}* DIG	1035.918	**<0.01**
CL= θ_1*EXP(ETA(1)) + θ_{26}* SUL	1047.867	>0.01
FULL MODEL		
CL= θ_1 *EXP(ETA(1)) + 0.0000000845 * AGE +0.000000717*CHOL+1.31*AST+0.00000101*AMI+ 115*DIG	985.943	

CL clearance (l/h); θ_1 typical value of CL; ETA (1) interindividual variability in CL; θ_2 to θ_{26} slopes of the covariate effects; TBW patient´s body weight (kg); SEX takes the value 1 for male and 0 for female; DD daily dose of clopidogrel (mg/day); ABCB1 genotype (C=wild type, T=increased P-glycoprotein activity); CYP2C19 phenotype (PM=poor metabolizer, IM=intermediate metabolizer, EM=extensive metabolizer, URM=ultrarapid metabolizer); CYP2C9 genotype (C=wild type, T=decreased activity); EF ejection fractions; CHOL cholesterol; TGL triglyceride; RBC red blood cells; AST aspartate transaminase; ALT alanine transaminase; CLcr creatinine clearance (l h^{-1}); DM presence of diabetes mellitus; TOB takes the value 1 for smokers and 0 for non-smokers; co-medication with PANT pantoprazole, BB beta blockers, DIU diuretics, SPI spironolactone, AML amlodipine, AMI amiodarone, STAT statine, DIG digoxin, SUL sulphonylureas takes the value 1 if the patient received co-medication and 0 otherwise.

** p-value for the MOF difference between the base and tested models

in Table 2. Required statistical significance (for $p<0.001$) in this step was the minimal difference in the MOF values of 6.63 between the base and a single regression model.

Thus, the full model consisted of the following covariates: age, cholesterol level, aspartate transaminase and co-medications with amiodarone and digoxin. Its effects on clearance of oxoclopidogrel had to be confirmed by the backward removal process with the strong statistical requirements (difference in MOF ≥10.83 for $p<0.001$). In the next table, marked as Table 3, we presented this process which resulted in the confirmation of two covariates with significant influence on the apparent clearance of oxoclopidogrel and its variability. These were the value of aspartate transaminase and co-medication with digoxin in the target population, described in the form of the equation as:

$$CL\ (lh^{-1}) = 1.7 + 1.31*\underline{AST + 115*DIGOXIN}$$

Table 3 The process of backward removal of covariate from a full PPK model of the clearance of oxoclopidogrel

Covariates	Difference in minimum objective function	p-value **
Age	1.798	>0.001
Cholesterol	1.557	>0.001
AST	50.084	**<0.001**
Amiodarone	0.969	>0.001
Digoxin	17.162	**<0.001**

** p-value for the MOF difference between the base and tested models

During the PPK analysis, the value of MOF was reduced by 61.924 units from the base to the final model. Furthermore, both inter-individual and residual variability (expressed as coefficient of variation) were 31.18% and 59.72% in the base model and were reduced to 16.01% and 41.51% in the final model, respectively. Much better correlation between measured oxoclopidogrel concentrations versus its population, predicted concentrations (ng/ml) in the final model is shown in Figure 1. The estimates of the apparent clearance of oxoclopidogrel, both investigated variability, and also the effects of aspartate transaminase and co-medication with digoxin, are confirmed by the bootstrap analysis indicating a good precision and the stability of the final model (Table 4).

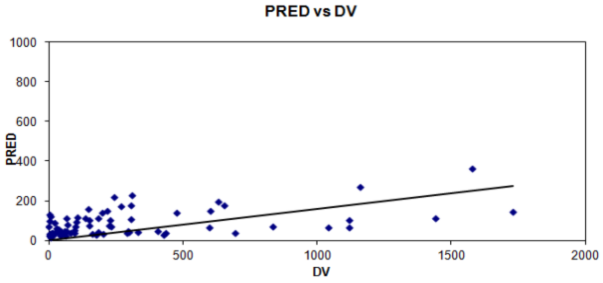

Fig. 1 Scatter-plot of predicted serum oxoclopidogrel concentrations (PRED) versus its measured serum concentrations (DV) expressed as ng/ml in the final model.

Discussion

In the present study we developed and used a population pharmacokinetic model for the clearance of 2-oxo-clopidogrel in routinely treated Serbian patients with ACS. We observed that the typical mean value for 2-oxo-clopidogrel clearance, estimated by the base model (without covariates) in our population was 39.2 l h^{-1}. Also, we noted large interindividual and residual variabilities in this phase.

Another potential source of pharmacokinetics variance of 2-oxo-clopidogrel is metabolic activity of CYP enzyme, especially the activity of CYP2C19 and CYP3A4 isoforms. The hepatic metabolism of 2-oxo-clopidogrel involves dominantly CYP219 and 3A4 isoforms, with 20.6 and 39,8% of contribution, respectively [7]. The interindividual variability in the enzyme activity is partially genetically determined. Among genetic factors, the impact of the following potential covariates was evaluated: CYP2C19*2, CYP2C19*17, and ABCB1 3435 TT genotype. Our findings showed that CYP2C19 genotypes did not influence the clearance of 2-oxo-clopidogrel. Additionally, clearance of 2-oxo-clopidogrel is not altered by ABCB1 3435 TT genotype. Future studies are required to verify these results, especially due to small sample size. The latter fact is an obvious limitation of this study, as well as only one sample per patient. Another PK analyses available in the literature are focused on the kinetics of inactive carboxylic acid metabolite [14, 15] or the H4 isomer which has the antiplatelet activity [16] and to the best of our knowledge no studies are available dealing with 2 oxo-clopidogrel kinetics.

Table 4 Parameter estimates in the final model of oxoclopidogrel

Parameter	NONMEM		Bootstrap Analysis	
	Estimate	95% CI*	Estimate	95% CI‡
CL/F (l/h)	1.7	1.17 – 2.23	1.66	1.05 – 2.27
AST	1.31	0.902 – 1.718	1.40	0.907 – 1.893
DIGOXIN	115.00	86.46 – 143.54	119.00	88.65 – 149.35
Interindividual variance of CL - ω^2_{CL}	0.0253	0.0154 – 0.0352	0.0261	0.0141 – 0.0381
Residual variance - σ^2 (exponential)	0.159	0.055 – 0.263	0.162	0.065 – 0.259

* (Estimate) ± 1.96 x (standard error of the estimate)
‡ 2.5th and 97.5th percentile of the ranked bootstrap parameter estimates

Supposing the possibility that concomitant administration of drugs which share the same CYP450 metabolizing isoenzyme may alter the systemic exposure of 2-oxo-clopidogrel, it was expected that any of them would have some influence on 2-oxo-clopidogrel. However, none of the drugs that are at least partially metabolized by CYP2C19 and 3A4 isoenzymes, influenced the clearance of 2-oxo-clopidogrel significantly. This could be explained by the relative contribution of additional CYP enzymes on 2-oxo-clopidogrel kinetics.

We tested the influence of 25 covariates on the pharmacokinetic disposition of 2-oxo-clopidogrel. In our final model, the only important factors influencing 2-oxo-clopidogrel clearance, from among the covariates tested, were the value of aspartate transaminase and co-medication with digoxin. Previous publications have suggested that both drugs (clopidogrel and digoxin) are substrates of P-glycoprotein (P-gp), but the coadministration of clopidogrel with digoxin did not impact the pharmacokinetics of clopidogrel [17]. However, our results clearly show that 2-oxo-clopidogrel CL is notably increased when digoxin is added to therapy. This was unexpected and the mechanism by which digoxin increases CL of 2-oxo-clopidogrel remains obscure.

The results of our study show that the value of aspartate transaminase significantly increases clearance of 2-oxo-clopidogrel. None of our patients had liver damage and their ALT values were in the normal range, or slightly above it. Its variability may be attributed to subtle myocardial damage in ACS. Additionally, the available information regarding correlation of liver test and the hemodynamic status is very scarce [18]. Therefore, future studies are required to verify these results.

Conclusion

Our PPK model for the clearance of 2-oxo-clopidogrel in routinely treated adult patients with ACS showed that the the value of aspartate transaminase and concomitant therapy with digoxin were the main subjects of 2-oxo-clopidogrel pharmacokinetic variability.

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References

1. Buonamici P, Marcucci R, Migliorini A, et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. J Am Coll Cardiol 2007; 49:2312–2317.
2. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol 2006; 48:1742–1750.
3. Sibbing D, Braun S, Morath T, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. J Am Coll Cardiol 2009; 53:849–856
4. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2007;357:2001–2015.
5. Wallentin L, Varenhorst C, James S, et al. Prasugrel achieves greater and faster P2Y12receptor-mediated platelet inhibition than clopidogrel due to more efficient generation of its active metabolite in aspirin-treated patients with coronary artery disease. Eur Heart J 2008; 29:21–30.
6. Karaźniewicz-Łada M, Danielak D, Burchardt P, Kruszyna L, Komosa A, Lesiak M, Główka F. Clinical pharmacokinetics of clopidogrel and its metabolites in patients with cardiovascular diseases. Clin Pharmacokinet 2014; 53:155–164.
7. Kazui M, Nishiya Y, Ishizuka T, et al. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos. 2010;38(1):92–99. doi:10.1124/dmd.109.029132.
8. Taubert D, Kastrati A, Harlfinger S, Gorchakova O, Lazar A, von Beckerath N, et al. Pharmacokinetics of clopidogrel after administration of a high loading dose. Thromb Haemost 2004; 92: 311−316. her 84, 236−242.
9. Elsinghorst PW. Quantitative determination of clopidogrel and its metabolites in biological samples: a mini-review. J Chromatogr B Analyt Technol Biomed Life Sci 2013; 917–918: 48–52.
10. Li X, Liu C, Zhu X, Wei H, et al. Evaluation of Tolerability, Pharmacokinetics and Pharmacodynamics of Vicagrel, a Novel P2Y12 Antagonist, in Healthy Chinese Volunteers. Front Pharmacol 2018; 9:643. doi: 10.3389/fphar.2018.00643. eCollection 2018.
11. Beal SL, Boeckmann AJ, Sheiner LB. NONMEM users guide. Parts I–VIII ICON Development Solutions 2013. Ellicott City, MD, USA.
12. Milovanovic JR, Jankovic SM. Population pharmacokinetics of lamotrigine in patients with epilepsy. Int J Clin Pharmacol Ther 2009; 47:752−760.
13. FDA (1999). Guidance for Industry Population Pharmacokinetics. FDA, Rockville. Available at: https://www.fda.gov/downloads/drugs/guidances/UCM072137.pdf (Last assessed: 10.12.2018.)
14. Yousef AM, Melhem M, Xue B, Arafat T, Reynolds DK, Van Wart SA. Population pharmacokinetic analysis of clopidogrel in healthy Jordanian subjects with emphasis optimal sampling strategy. Biopharm Drug Dispos 2013; 34(4):215−226. doi:10.1002/bdd.1839.
15. Lee J, Hwang Y, Kang W, et al. Population pharmacokinetic/pharmacodynamic modeling of clopidogrel in Korean healthy volunteers and stroke patients. J Clin Pharmacol 2012; 52(7):985−995. doi:10.1177/0091270011409228
16. Danielak D, Karaźniewicz-Łada M, Komosa A, Burchardt P, Lesiak M, Kruszyna Ł, Graczyk-Szuster A, Główka F. Influence of genetic co-factors on the population pharmacokinetic model for clopidogrel and its active thiol metabolite. Eur J Clin Pharmacol 2017; 73(12):1623–1632. doi: 10.1007/s00228-017-2334-z.
17. Peeters PA, Crijns HJ, Tamminga WJ, Jonkman JH, Dickinson JP, Necciari J. Clopidogrel, a novel antiplatelet agent, and digoxin: absence of pharmacodynamic and pharmacokinetic interaction. Semin Thromb Hemost 1999; 25 Suppl 2:51−54.
18. Van Deursen VM, Damman K, Hillege HL, van Beek AP, van Veld-huisen DJ, Voors AA. Abnormal liver function in relation to hemodynamic profile in heart failure patients. J Card Fail 2010; 16:84–90.