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CARVEDILOL POPULATION PHARMACOKINETIC ANALYSIS – APPLIED VALIDATION PROCEDURE

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Carvedilol is a nonselective beta blocker/alpha-1 blocker, which is used for treatment of essential hypertension, chronic stable angina, unstable angina and ischemic left ventricular dysfunction. The aim of this study was to describe carvedilol population pharmacokinetic (PK) analysis as well as the validation of analytical procedure, which is an important step regarding this approach. In contemporary clinical practice, population PK analysis is often more important than standard PK approach in setting a mathematical model that describes the PK parameters. Also, it includes the variables that have particular importance in the drugs pharmacokinetics such as sex, body mass, dosage, pharmaceutical form, pathophysiological state, disease associated with the organism or the presence of a specific polymorphism in the isoenzyme important for biotransformation of the drug. One of the most frequently used approach in population PK analysis is the Nonlinear Modeling of Mixed Effects - NONMEM modeling. Analytical methods used in the data collection period is of great importance for the implementation of a population PK analysis of carvedilol in order to obtain reliable data that can be useful in clinical practice. High performance liquid chromatography (HPLC) analysis of carvedilol is used to confirm the identity of a drug and provide quantitative results and also to monitor the efficacy of the therapy. Analytical procedures used in other studies could not be fully implemented in our research as it was necessary to perform certain modification and validation of the method with the aim of using the obtained results for the purpose of a population pharmacokinetic analysis. Validation process is a logical terminal phase of analytical procedure development that provides applicability of the procedure itself. The goal of validation is to ensure consistency of the method and accuracy of results or to confirm the selection of analytical method for a given sample and drug. This study confirmed the importance of using valid analytical procedure for the purpose of carvedilol population pharmacokinetic analysis. Identification of demographic, pathophysiological and other factors that may influence the population carvedilol PK parameters gives the physician the possibility of a more comprehensive overview of the patient and better optimization of the therapeutical regimen. *Acta Medica Medianae 2013;52(3):18-24.*

Key words: *carvedilol, population pharmacokinetic, HPLC, validation*

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Introduction

In the recent years, the use of β-blockers (especially for uncomplicated hypertension) has been widely debated. Carvedilol is a nonselective beta blocker/alpha-1 blocker, who expresses vasodilatator effects (1). It binds to the receptors, inhibits their action and causes the blood vessels dilatation. Additionally, carvedilol is a potent antioxidant and anti-proliferative agent. Carvedilol reduces the peripheral vascular resistance through vasodilatation and blocking the renin-angiotensin-aldosterone system. Carvedilol decreases blood pressure as well as heart rate, while the renal blood flow and renal function are preserved in patients with arterial hypertension. Cold extremities, which is often an adverse effect of the beta – blockers, are rare during carvedilol usage due to preserved blood flow through the extremities as well (2). It is used to treat essential hyper-tension, chronic stable angina, unstable angina and ischemic left ventricular dysfunction. Carvedilol is used to treat congestive heart failure as it leads to reduction in mortality, rate of hospitalization, and improvement of disease progression. Possible side effects that could be expressed during carvedilol treatment include blurred vision, headaches, fatigue, brady-cardia, postural hypotension, hypotension, edema, nausea, diarrhea, vomiting, thrombocytopenia, hyperglycemia,

weight gain, hypercholesterolemia, asthma and dyspnea in patients with a predisposition, pain in the extremities and decreased lacrimation.

Chemically, carvedilol is a racemic mixture of S and R enantiomers, of which the S enantiomer is responsible for the blockade of beta receptors (3). Carvedilol is characterized by distinct lipophilicity, which underlies the rapid absorption from the gastrointestinal tract. Maximum plasma concentration of carvedilol is being achieved in 1-2 hr after oral drug administration. It shows high rate of first-pass metabolism and high percentage of plasma proteins binding as well. It is a substrate for microsomal liver isoenzymes, especially cytochrome P450 2D6 (CYP 2D6), but cytochrome P450 2C9 (CYP 2C9), 3A4 (CYP 3A4), 1A2 (CYP 1A2), 2C19 (CYP 2C19) and 2E1 (CYP 2E1) are also involved in the biotransformation of carvedilol (3). Elimination half-life is 6 to 10 hours, which allows dosing every 12 hours. Participation of CYP 2D6 in the metabolism of carvedilol causes a large inter-individual variability in the pharmacokinetics (PKs) and pharmacodynamics (PDs) of drug due to expressed polymorphism. CYP 2D6 enzyme gene polymorphism implies the existence of fast and slow metabolizers, which may determine variability in carvedilol clearance. This could lead to adverse drug effects or lack in efficiency after the application of the usual dose.

Analytical methods need to be modified and validated in order to be used in other studies of the PK population. The goal of validation is to ensure consistency of the method and accuracy of results or to confirm the selection of analytical method for a given sample and drug (4). The main criteria are that the analytical method should meet are accuracy and precision (5). Validation process is a logical terminal phase of analytical procedure development that provides applicability of the procedure itself. Revalidation is always done when the existing methods is going to be used in other laboratory or when changes occur in the analytical procedure or the substance itself.

Accordingly, for the investigation of the pharmacokinetics of carvedilol, we have developed a simple, sensitive method for its determination in human plasma. Regarding the period of the data collection for the population PK analysis, the total number of data that need to be processed and the complexity of population PK analysis procedure itself, it is necessary to validate the analytical procedure for carvedilol determination due to correct interpretation of the analysis results. Therefore, the aim of this study was to describe carvedilol population pharmaco-kinetic analysis as well as the validation of analytical procedure for its determination in human plasma samples.

Previous studies on carvedilol HPLC determination

High performance liquid chromatography (HPLC) is most frequently used for carvedilol determination in human plasma samples. This analytical method has also significant use in PK studies (6). For instance, PK studies of carvedilol after administration of buccal patch in pigs also applied the HPLC analysis. In this study, carvedilol and internal standard (Flecainide acetate) were extracted into n-hexane-dichloromethane solvent system and separated using an isocratic mobile phase on a Phenomenex C (18) column (7). The mobile phase consists of acetonitrile, methanol, water, and triethylamine at a ratio of 25:20: 54.9:0.1 (v/v). The eluent was monitored by spectroflourimetric detector at a flow rate of 1.0 mL/min. The pH was adjusted to 2.5 with orthophosphoric acid. Ptacek et al. introduced the HPLC method as simple and highly sensitive, based on protein precipitation with methanol and concentration of the supernatant by evaporation and reversed - phase chromatography with fluorimetric detection (8). The separation was performed on a Develosil 3 mm ODS 10034.6 mm I.D. column and the mobile phase consisted of acetonitrile - 30 mM potassium dihydrogenphosphate buffer, pH 2 (30:70 v/v). This method was further developed and validated for pharmacokinetics study by Zarghi et al. (9). Talebpour et al. recommended an extraction method for determination of carvedilol in human plasma samples, employing stir bar coated with a film of polymethyl metacrylate/ethylenglycol dimethacrylate and polymimethyl siloxane in combination with liquid desorption, followed by HPLC with ultraviolet detection (10). Due to specificity of our conditions, our analysis required changes in the application of already validated HPLC methods. The development and the validation of HPLC method for carvedilol determination in human plasma showed that our method had satisfactory characteristics for its application in pharmacokinetic purposes.

Material and methods

Reagents and chemicals

Standard of carvedilol was obtained from Sigma-Aldrich (GmbH Germany). HPLC grade methanol was supplied by J.T. Baker (Avantor Performance Materials, Deventer, The Netherlands). Potassium dihydrogen phosphate and potassium hydroxide of analytical grade were obtained from Merck (Darmstadt, Germany).

HPLC conditions and analysis

The chromatographic analysis was performed with an Agilent liquid chromatograph RR HPLC 1200 (Agilent Technologies, Santa Clara, CA, USA) using a fluorescent detector. The analytical column was Zorbax SB-C18, 4.6 x 150 mm i.d, 3.5 μm particle size (Agilent Technologies, Santa Clara, CA, USA). Dihydroergocristine mesylate was used as the internal standard. The mobile phase consisted of 30 mM potassium dihydrogen

phosphate buffer adjusted to pH 2.3 with KOH (mobile phase A) and methanol (mobile phase B). Gradient elution was performed at a flow rate of 1.5 mL/min starting with 48% mobile phase A and 52% mobile phase B and keeping that composition for the next 3 minutes; from 3 to 3.5 min gradient changed to 100% mobile phase B remaining the same to 6.5 min; from 6.5 to 7.0 min gradient returned to starting condition staying the same till 8 min, which was the end of separation.

Total analysis time was 10 min with the 2 min of equilibration time. The detection was performed fluorometrically with an excitation wavelength of 240 nm and an emission wavelength of 352 nm with the gain of 10, except between 2.85 and 3.40 min when it was 12. All separations were carried out at the temperature of 40 °C.

Standard and calibration solutions

The stock solution of carvedilol was prepared in methanol in a concentration of 1 mg/mL. Working solutions (10 ng/mL, 100 ng/mL and 1000 ng/mL) are diluted with the mobile phase A. Calibration solutions for carvedilol were prepared by mixing appropriate amounts of working solution with blank plasma to obtain concentrations of 1, 5, 10, 25, 50 and 100 ng/mL.

Sample preparation for HPLC analysis

For sample preparation 600 µL of cold methanol was added to 200 µL of plasma sample. This was followed by vortexing and centrifugation step at 12000 rpm for 10 minutes at 4°C. Afterwards, 550 µL of the supernatant was collected and 20 µL was injected into HPLC system.

Population pharmacokinetics approach of carvedilol

Population PK analysis of carvedilol is carried out using the substance plasma concentrations which have been collected over a longer period of time from a large number of patients. Patients and this data before analysis were divided into two groups - group for the population mathematical model building as well as group for the validation of this model.

One of the most frequently used approaches in population pharmacokinetics (PK) analysis is the Nonlinear Modeling of Mixed Effects - NONMEM modeling. It is considering two levels of effects - fixed effects that define the typical population values of PK parameters and random effects showing the range of possible values. The data required for this type of PK analysis are being collected during routine medical control of the patients, which is an advantage of this approach. Alternatively, processing of the collected data is a very complex process that involves application of algorithms and software with the task to create appropriate mathematical model of the chosen system. Regarding choosing the software for population analysis, it is necessary to know the PK parameters of the tested drug in order to have accurate modeling. Procedure for the population PK analysis has three primary phases. The first phase defines the base model, which does not include covariates. This model gives us typical values of population pharmacokinetics parameters, such as drug clearance and the volume of distribution. Also, it defines covariates for interindividual and intraindividual variability. The second phase is more complex and represents a stepwise process in building univariate regression models (11,12). The evaluation of statistical significance of every variable follows the reduction in the minimum value of objective function (MOF) between univariate models. The final step of this phase includes more restrictive criteria and deletion of some variables to get adequate final model. Also, it defines error model and mentioned covariate is added in the mathematical model (3). When the final model is created, which includes significant variables, it is necessary to verify the obtained model. The third phase is validation of the model. Validation group of patients and date set were not included in the process of building final model. The process of validation confirmed the correctness of the obtained model and enables it to be used for clinical purposes.

Validation of HPLC procedure for carvedilol

Validation and revalidation of the method for quantitative analysis of drugs include determining the selectivity, accuracy, precision, linearity, LOD and LOQ. Validation procedure was carried out using the standard of carvedilol.

The selectivity of HPLC method was confirmed by injecting blank samples (from healthy volunteers, without carvedilol), spiked samples and standard solutions. The linearity of an analytical procedure is its ability (within a given range) to obtain measured values which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of the method was estimated by the regression equation.

The limit of detection (LOD) was measured as the lowest amount of carvedilol detected with response significantly different from that of a blank. The limit of quantification (LOQ) was obtained as 3.3 time higher concentration of the analyte compared to LOD.

The accuracy shows deviation of results obtained by the chosen method from the reference values whereas the precision is the closeness of agreement of the results in a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method must be defined at three levels: repeatability, intermediate precision and repro-

ducibility. The precision and accuracy of analytical method were investigated using samples containing known amounts of the carvedilol. They were measured using five replicate injections for three quantification levels (QC levels): low QC (4 ng/mL), medium QC (40 ng/mL) and high QC (80 ng/mL).

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity (4). Defining the range of the chosen measurement, the method can achieve satisfactory precision, linearity and accuracy, which is important for the interpretation of results and PK analysis.

This study was approved by the Ethics Committee of the Faculty of Medicine (No: 01-5413-4).

Results

The HPLC method was tested for selectivity, linearity, limit of detection, limit of quantification, precision and accuracy.

The study of selectivity showed that there were no interfering substances at the retention time of carvedilol (3.12 min). Figure 1 shows three chromatograms with the retention times of different samples: A – Sample of a carvedilol standard, B – Control Sample, C – Real Sample.

The control sample contained the blood of a person who did not use carvedilol.

The linear dependencies were established between the response (peak area) and carvedilol concentration over the tested range from 1–100 ng/mL. The regression equation Y=7.2753x + 10.0277 and correlation coefficient R^2=0.9994 were achieved.

The limit of detection and quantification for carvedilol (LOD and LOQ) were 0.3 ng/mL and 1.0 ng/mL, respectively.

Determined mean precision for three QC levels, expressed as relative standard deviation (RSD, %), was 3.73%. The accuracy of the method was reported as recovery value (R, %). The obtained R for low QC, medium QC and high QC was 85.33%, 98.22% and 99.10%, respectively. The between - day precision was also determined. The results for between - day precision showed that relative standard deviation was less than 6.0 %.

Discussion

The results of validation confirmed that the chosen HPLC method for carvedilol determination in chosen HPLC conditions showed good selectivity and wide range of linearity with appropriate coefficient of correlation. LOD and LOQ of our method have the low values, which provide determination of very low concentration of carvedilol in the human plasma. Also, relative standard deviation and recovery value showed satisfactory accuracy and precision of carvedilol determination in chosen type of sample. The obtained results of the validation confirmed validity of data for the application in NONMEM software in order to determine population PK parameters of carvedilol.

In contemporary clinical practice, population PK analysis is often more important than standard PK approach in setting a mathematical model that describes the PK parameters and takes into account the variables that have particular importance in the drugs pharmacokinetics. Possible variables that can influence population PK parameters are sex, body mass, dosage, pharmaceutical form, pathophysiological state, disease associated with the organism or the presence of a specific polymorphism in the isoenzyme important for biotransformation of the drug.

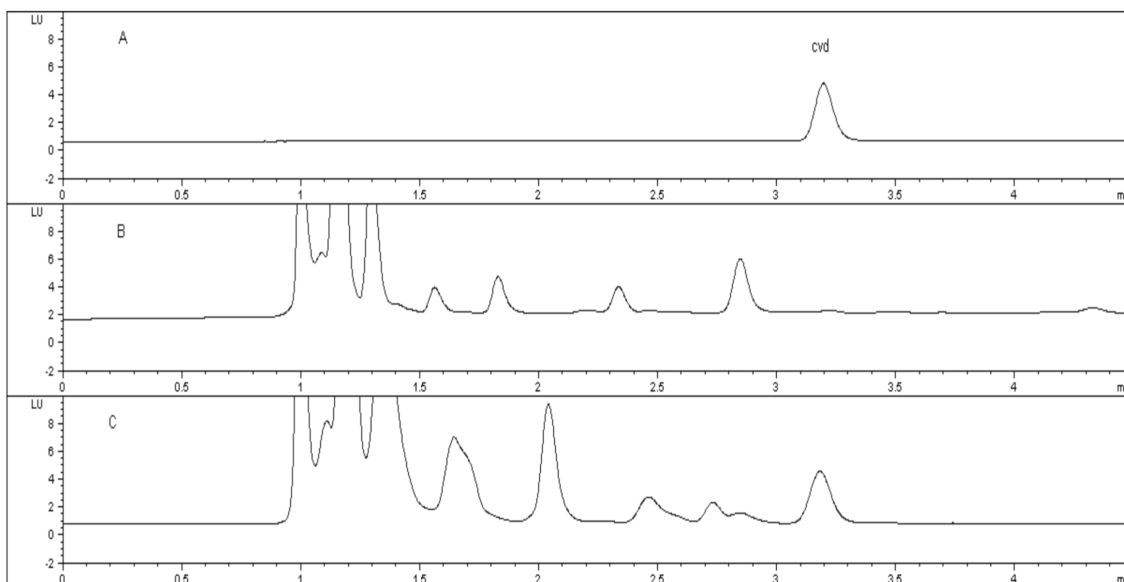

Figure 1. Retention times of carvedilol samples: A - Standard of Carvedilol, B - Control sample, C - Real sample

Data, including concentration, sampling time and time elapsed since the last taken dose need to be modeled appropriately using the software, which in the first phase defines the basic model of the system. The variables are evaluated based on their impact on the value of population parameters of a drug, which leads to identification of significant variables that are included in the final population pharmacokinetic model of the PK parameters.

Results of population pharmacokinetics carvedilol aproach suggest that the body weight, concomitant therapy with digoxin, and tobacco using are the main subjects of his PK variability (13,14). The study population consisted of 52 Caucasian patients with chronic heart failure, receiving carvedilol therapy. Population PK approach was performed with NONMEM software, ADVAN2, TRANS2 procedure for building base model. This means one-compartment model with first order elimination rate (15). In some PK studies the authors showed a significant effect of CYP2D6 isoenzyme of in carvedilol clearance within Japanese and Caucasian populations (16). Owing to its characteristics, population PK analysis provides new and important information on the product and allows obtaining clinically relevant data on the pharmacokinetics of the drug in the target study population.

Identification of demographic, pathophysiological and other factors that may influence the population carvedilol PK parameters gives the physician the possibility of a more comprehensive overview of the patient and better optimization of the therapeutical regimen (13,16).

In conclusion, validation process itself justifies the scientific value of established modifications, providing the application of the obtained results for the purpose of PK analysis. Professional and multidisciplinary approach in the interpretation of the obtained results provided the access to specific population PK parameters of carvedilol. Population analysis of carvedilol showed body weight, smoking, and the presence of digoxin as the variables that may influence the population PK parameters of carvedilol in patients with congestive heart failure. This specificity provides a better therapeutic approach to the patients by physician, who always use PK parameters as important guidelines in setting up and modification of the treatment regimen.

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VALIDACIJA ANALITIČKE PROCEDURE KARVEDILOLA ZA PRIMENU U POPULACIONOJ FARMAKOKINETIČKOJ ANALIZI

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Karvedilol je neselektivni beta/alfa-1 blokator koji se koristi za lečenje hipertenzije, hronično stabilne angine i nestabilne angine pectoris, kao i ishemijske disfunkcije leve komore. Cilj ovog rada bio je da se opiše postupak populacione farmakokinetičke (FK) analize karvedilola, kao i da se naglasi važnost validacije analitičke procedure. U savremenoj kliničkoj praksi, populaciona FK analiza često je značajnija od prostorne FK analize kao standardne procedure. Ona postavlja matematički model koji opisuje FK parametre. Pored toga, populacioni FK model uključuje varijable koje imaju poseban značaj za farmakokinetiku datog leka, kao što su: pol, telesna težina, doza leka, farmaceutski oblik, patofiziološko stanje, pridružene bolesti, kao i prisustvo odgovarajućeg fenotipa izoenzima koji učestvuje u metabolizmu leka. Jedan od najčešće korišćenih pristupa u populacionoj FK analizi je nelinearno modeliranje mešovitih efekata - NONMEM modeliranje. Analitičke metode za prikupljanje podataka od velikog su značaja za pravilno populaciono FK modelovanje karvedilola. Visoko-efikasna tečna hromatografija koristi se u kvalitativne i kvantitativne svrhe i služi za dobijanje pouzdanih podataka, koji bi bili od koristi u kliničkoj praksi za praćenje efikasnosti terapije. Analitičke procedure korišćene u drugim studijama nisu mogle biti u potpunosti prenešene na naše istraživanje, već je bilo neophodno izvršiti modifikaciju i validaciju metode u cilju korišćenja dobijenih rezultata za populacionu farmakokinetičku analizu. Validacija je logičan završni proces u razvoju analitičkog postupka i omogućava primenljivost samog postupka. Cilj validacije je da obezbedi konzistentnost metode i tačnost rezultata, odnosno da potvrdi izbor analitičke metode za dati uzorak i lek. Ova studija je potvrdila važnost korišćenja validiranog analitičkog postupka u populacionoj FK analizi karvedilola. Određivanje demografskih, patofizioloških i drugih faktora koji mogu da utiču na populacione FK parametre karvedilola daju mogućnost lekaru da sveobuhvatnije sagleda bolesnike i omogući optimizaciju terapijskog režima. *Acta Medica Medianae 2013; 52(3):18-24.*

Ključne reči: *karvedilol, populaciona farmakokinetika, HPLC, validacija*

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