Department of Pharmacy[1], Faculty of Medicine; Faculty of Mechanical Engineering[2], University of Nis, Nis; Department of Pharmacology, Toxicology and Clinical Pharmacology[3], Faculty of Medicine, University of Novi Sad, Novi Sad; Clinic of Nephrology[4], Clinical Center Nis, Nis, Serbia

Evaluation of gender-based limited sampling methods for tacrolimus exposure after renal transplantation using the Monte Carlo simulation

A. Catić-Đorđević[1], I. Pavlović[2], D. Pavlović[1,*], N. Stefanović[1], M. Mikov[3], T. Cvetković[1,4], R. Veličković-Radovanović[1,4]

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**Corresponding author: Dragana Pavlović, Department of Pharmacy, Faculty of Medicine, University of Nis, Nis, Serbia*
dragana.pavlovic@medfak.ni.ac.rs

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Numerous adverse and toxic effects of tacrolimus (Tac) are linked with a marked variability in its pharmacokinetics. New focus in this area is finding optimal measuring concentration points of Tac in order to predict area under the concentration-time curve (AUC). Currently, there is no consensus on the optimal strategies for the best AUC predictable concentrations. Potential introduction of the Monte Carlo (MC) method in clinical investigations is very important in the assessment of the most expecting numerical results for vulnerable transplant patients according to the derived analytical models. The study aimed to evaluate the best predictable concentration of Tac in order to predict body drug exposure after renal transplantation performing MC simulation with respect to gender. First part was presented as experimental measurements of Tac as well developed mathematical models for correlation of AUC and Tac concentrations. The second part was application of MC method in order to calculate AUC and compare new obtained results with previous data. For optimal determination of the AUC in female population: according to regression model the samples should be taken after two and twelve hours, while MC simulation suggested that sampling should be done after one and eight hours. In male population, eight hours after administration was the best sampling time according to both, regression and MC. The findings of MC simulation emphasized the gender differences in Tac pharmacokinetics and gender-based clinical approach. Monte Carlo simulation approach suggested two sampling points in female patients, one and eight hours, and one sampling point in male patients, eight hours after oral administration, for prediction of Tac exposure in renal transplant recipients.

1. Introduction

Renal transplantation is an optimal solution for patients with end-stage renal disease. It ensures prolonged survival and better recipient's quality of life compared to dialysis. Long-term graft survival after renal transplantation relies significantly on proper choice of immunosuppressive protocol and its management (Thölking et al. 2017; Sienkiewicz et al. 2016). Since its first approval by the Food and Drug Administration in 1994, tacrolimus (Tac) is widely used as immunosuppressant in solid organ transplantation notwithstanding its numerous adverse effects, neuro- and nephrotoxicity, a narrow therapeutic index and marked inter-individual pharmacokinetic variability (Brooks et al. 2016; Bouamar et al. 2013). In addition to the well-known inter-patient variability, achieving optimal dosage regimen of Tac is also complicated by intra-patient variability in Tac exposure, which demands immunosuppressive therapy individualization (Shuker et al. 2015). Tacrolimus elimination is mainly affected by CYP-mediated metabolic biotransformation and P-glycoprotein (P-gp) efflux, which increase already high potential for drug and food interactions (Riegersperger et al. 2016, Stefanović et al. 2015). The increased variability in Tac concentrations is linked with graft rejection (Huang et al. 2016). Therefore, after steady state is achieved, routine therapeutic drug monitoring (TDM) and parallel biochemical monitoring are required steps to improve treatment outcomes in the post-transplant period (Velickovic-Radovanovic et al. 2012). Therapeutic drug monitoring is mandatory for most immunosuppressants and has become an integral part of immunosuppressive drug therapy management. It is usually based on trough concentration (C_0) monitoring, but other TDM tools include the area under the concentration-time curve (AUC) over the (12-hour) dosage interval as parameter of body drug exposure, adverse effects and toxicity (Scholten et al. 2015; Musuamba et al. 2013; Monchaud et al. 2009). Consequently, new focus of pharmacokinetic research in this area is finding optimal measuring concentration points in order to predict AUC. Regardless, there is no current consensus on the optimal strategies for the best AUC predictable concentrations (Velickovic-Radovanovic et al. 2015; Vadcharavivad et al. 2016).

Our previous study showed that gender might represent an important factor in order to determinate the sampling time for best predictable Tac concentration for AUC. According to our results, upon reaching steady state, in female recipients concentration two hours after administration is assumed to be the best predictable. In male recipients, concentration eight hours after administration seemed to be a good indicator of total body exposure to Tac. Therefore, this novel study is focused on clarification the most predictable Tac concentration for AUC in relation to gender by applying the Monte Carlo (MC) simulation method. Monte Carlo method is very important in assessing the validity of the approximate analytical results. This particular approach finds great application in different applied sciences, including medicine, for prediction of many diverse parameters of interest. This method is widely used in every field of study and presents a broad class of computational algorithms that rely on repeated random sampling to obtain numerical results (Shasha et al. 2015). Also, application of this method on appropriate mathematical models and numeric simulations in clinical investigations means avoiding frequent invasive sampling in order to estimate pharmacokinetic parameters (e.g. AUC) which is an advantage for vulnerable patients. Nowadays, the MC method is successfully applied for antibiotic therapy optimization (Zhou et al. 2017; Kato et al. 2017). Besides, this method is useful tool for pharmacoeconomic calculations. There were efforts for the implementation of MC in Tac

research in the past, but they mostly dealt with pharmacoeconomic or pharmaceutical technology problems (Orme et al. 2003; Poole et al. 2010). This is a study on the application of the MC method in order to predict body drug exposure and management of adverse effects of Tac. The study aimed to evaluate the best predictable concentration of Tac in order to predict body drug exposure in renal transplant recipients performing MC simulation with respect to gender.

2. Investigations and results

2.1. Experimental study and modeling

Our previous experimental measurements of Tac concentrations were performed at the Clinic of Nephrology in Clinical Center Nis, Serbia (Velickovic-Radovanovic et al. 2015). After steady state was achieved, Tac daily dose was adjusted in accordance with the level of Tac, with drug concentration maintained in the optimal range (5-15 ng/mL). Blood concentrations were measured by microparticle enzyme immunoassay method. Non-compartmental pharmacokinetic analysis included calculation of AUC (0-12) for each patient in the steady state from a plot of Tac concentration versus time from time of drug administration to 12 h after administration, using the trapezoid rule. Associations between each sampling time point of concentrations within 12 h after the *per os* administration of Tac and AUC (0-12) were evaluated by Pearson correlation coefficients. Abbreviated sampling equations were derived by multiple stepwise regression analyses performed using AUC (0-12) as the dependent variables. Statistically significant difference was not found in AUC (0-12) between male and female patients in a steady state. Figure 1 shows measured concentrations of Tac in different time after oral administration in renal transplant recipients in steady state.

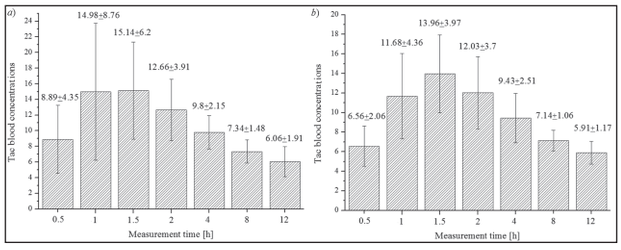

Fig. 1: Experimental Tac measurements with standard deviations for a) female patients and b) male patients

Regression analysis provided a system of equations to create a mathematical model with Tac concentrations in different sampling time as independent variables and predicted AUC (0 –12) (AUCp) as dependent variable. The variance in the strength of association between predicted AUCp and AUC (0 –12) was reflected by linear regression coefficients of multiple determinations. Data are shown as correlation coefficients (r), and coefficient of determination (r^2). According to experimental study the following models for AUCp determination are suggested:

Equations	r	r^2	
$AUCp=-0.427+14.289C_8$	0.905	0.819	(1)
$AUCp=-3.999+12.751C_8+1.104C_1$	0.979	0.959	(2)
$AUCp=-0.509+10.655C_8+0.775C_1+1.301C_2$	0.987	0.975	(3)
$AUCp=-0.452+7.608C_8+0.857C_1+1.503C_2+1.914C_4$	0.998	0.995	(4)
$AUCp=2.356+5.471C_8+0.833C_1+1.582C_2+2.295C_4+1.394C_{12}$	0.998	0.997	(5)
$AUCp=0.573+4.225C_8+0.679C_1+1.581C_2+2.694C_4+2.344C_{12}+0.433C_{0.5}$	0.999	0.999	(6)
$AUCp=0.000000497+4C_8+0.5C_1+1.25C_2+3C_4+2C_{12}+0.5C_{0.5}+0.5C_{1.5}$	1.000	1.000	(7)

where $C_{0.5}$, C_1, $C_{1.5}$, C_2, C_4, C_8 and C_{12} are Tac concentrations measurements after 0.5, 1, 1.5, 2, 4, 8, 12 h respectively.

Besides data, previously presented by Velickovic-Radovanovic et al., using gender-based regression analysis and calculation of AUC, the following model equations were obtained:

Equations	r	r^2	
$AUCp=-4.442+14.602C_8$	0.926	0.858	(8)
$AUCp=26.309+3.494C_2+5.881C_{12}$	0.985	0.969	(9)

where AUC models presented by equation (8) and (9) are given for male and female group respectively.

Numerical approaches were used to evaluate the most optimal AUC value in order to reveal the best model given by Eqs. (1) to (7) for all renal recipients and (8) to (9) for the equations in relation to gender. The numerical approach is based on expanding the exact solution of the system. The models for AUC determination given by Eqs. (1) to (7) are firstly tested and each other compared by performing the MC experiments using MATLAB (8.3.0.532) software.

According to measured values obtained in this clinical study for Tac applied on derived models, the expected value of AUC was numerically determined after N=10000 calculations.

According to the MC method the numerical experiments were obtained for the N experiments and AUC calculations using the state vector AUC = ($C_{0.5}$,C_1, $C_{1.5}$, C_2, C_4, C_8, C_{12}),

$$AUC_k=0.000000497+4C_{8k}+0.5C_{1k}+1.25C_{2k}+3C_{4k}+2C_{12k}+0.5C_{0.5k}+0.5C_{1.5k} \quad (10)$$

where k denotes the iteration the $C_{0.5k}$, C_{1k}, $C_{1.5k}$, C_{2k}, C_{4k}, C_{8k} and C_{12k} are the random variables from k-th iteration obtained from the performed experimental study and calculated standard deviations of these values.

2.2. Results

Following the MC numerical simulation of AUC models given by equations numbered from (1) to (7) the results for female, male and all renal recipients are presented in Fig. 2.

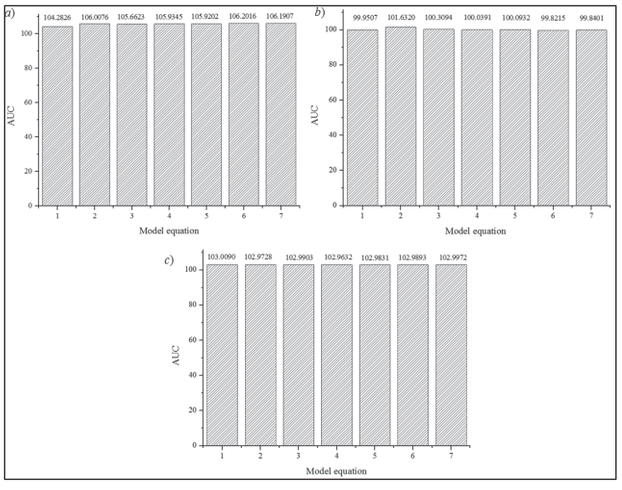

Fig. 2: Optimal values of AUC obtained using Monte Carlo simulation method for a) female patients, b) male patients and c) all tested patients

According to Fig. 2 a and b, the most appropriate models as representative models for AUC determination are chosen. So, by observing the models which deviation around the average calculated AUC values are the least and taking into account the number of taken samples, the model represented by Eq. (2) was adopted as a main model for female patients AUC determination. Similarly, for male population the simplest model given by Eq. (1) was chosen.

Further, using the MC simulation method the numerical results obtained from Eqs. (8) and (9) were calculated and compared with adopted models for male and female population, respectively. Results are presented in Figs. 3 and 4.

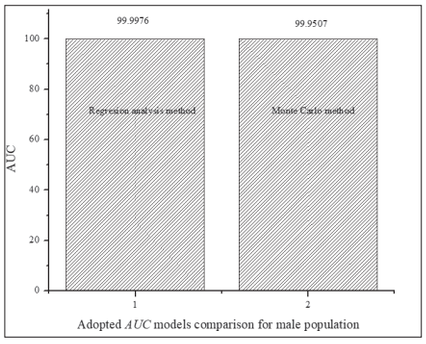

Fig. 3: Comparison of regression analysis results (1) and results obtained from Monte Carlo simulation (2) for female patients

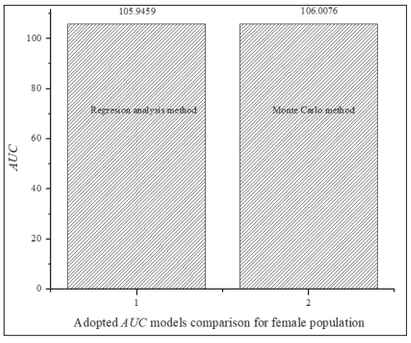

Fig. 4: Comparison of regression analysis results (1) and results obtained from Monte Carlo simulation (2) for male patients

Figures 3 and 4 showed the obtained results by regression analysis and by MC numeric simulations with minimal differences.
Also, the obtained MC model was used to calculate Tac clearance (CL_{Tac}), as an example of its clinical significance (Fig. 5).

3. Discussion

Bearing in mind that the 12-hours AUC is associated with adverse reactions and toxicity of Tac, prediction of AUC levels could be useful for better safety of transplant patients considering its inter- and intraindividual pharmacokinetic variability (Bessa et al. 2016). Previous studies showed that AUC might be a better predictor of Tac exposure and safety than monitoring of its trough concentration. The monitoring of AUC can achieve better therapy outcomes and control of adverse effects, such as post-transplant diabetes mellitus. This is a simple method for predicting body drug exposure that provides the opportunity to tailor immunosuppression and minimize tacrolimus-associated toxicity. Finding the best predictable concentration of Tac, which adequately represents AUC has been of great significance for previous and ongoing research. For example, Chen et al. (2005) found concentration 5 hours after the oral administration (C5) of Tac as the best predictable concentration for AUC and clinical outcomes. According to many authors, routine clinical practice to measure trough concentration of Tac before morning oral administration in steady state, was less reliable than measuring two concentrations (Ling et al. 2014; Niioka et al. 2013; Op den Buijsch et al. 2007). Vadcharavivad and al. (2016) suggested that a two-point sampling method of concentration measuring at 2 and 4 h after administration might have been a more cost-effective Tac monitoring strategy with low bias and high precision in predicting the AUC 0-12 in kidney transplant patients (Vadcharavivad et al. 2016). Generally, limited sampling methods that require inclusion of a concentration measurement from >2 h post-dose better predict Tac exposure than C(0) measurement (Barraclough et al. 2011). The Monte Carlo method was introduced to increase the confidence in the chosen measurement time for Tac AUC calculations. The virtual increase in the number of renal transplant recipients by generating random numbers in the range of standard deviation of the experimental measurements for different time sampling was done for that reason. There are very small variations in determined AUC values for male and female populations using regression analyzing and MC method. Therefore, the comparison of results verifies the findings of our previous research (Velickovic-Radovanovic et al. 2015). Thus, for the optimal determination of the AUC in female population: according to the regression model the samples should be taken after two and twelve hours, while MC simulation suggested that sampling should be done after one and eight hours. In male population, eight hours after administration was the best sampling time according to both, regression and MC. Considering the difference in female patients between regression model and MC simulation, dosage adjustment should be done according to the MC approach. This recommendation arose from a larger number of patients in MC simulation compared to regression analysis. In accordance to previous studies this approach suggested the avoidance of minimal concentration in 12 h dosing interval for dosage adjustments (Vadcharavivad et al. 2016; Chen et al.2005; Barraclough et al. 2011). These results confirmed reality of noncoherence in the available results, suggesting different concentrations for optimal AUC prediction. In relation to gender differences in Tac pharmacokinetics, and based on the previous studies, this research suggested the necessity of two sampling times at the beginning and the end of a dosing interval for female patients and one sampling time at the end of dosing interval for male patients.

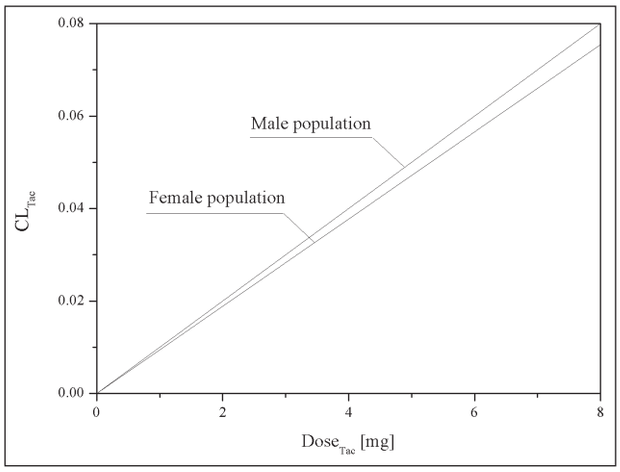

Fig. 5: Dependency of tacrolimus clearance related to gender

In conclusion, this study showed that implementation of the MC method in pharmacokinetic analysis and drug concentration prediction may be a useful approach in treating renal transplant recipients. As one of the pioneers in introduction of MC method into Tac pharmacokinetic calculations, this numerical simulation increased confidence in prediction of pharmacokinetic parameters considering a large number of numerical MC patients. This approach suggested two sampling points, one and eight hours, in female patients and one sampling point, eight hours, in male patients in prediction of Tac exposure after renal transplantation. The obtained findings of MC simulation emphasized the gender differences in Tac pharmacokinetics and therefore gender-based clinical approach, which was showed for Tac clearance.

4. Experimental

The method of this investigation is organized as follows. First part is presented as experimental measurements of Tac as well as developed mathematical models for correlation of AUC and Tac concentrations. In the second part of the study MC method was applied in order to calculate AUC and compare new obtained results with previous data from mathematical model.

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