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Computer-based simulation of plasma concentration time-profiles of drug in nonlinear two-compartment model

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The main interest of pharmacokinetics is the study of the fate of drugs in the living organism. This work proposes the system of the conservation laws that describes time-dependent concentrations of a drug, after a single intravenous administration. Compared with others, the proposed model considers both free and protein-bound drug concentrations at the same time. Plasma protein binding captured in the model enters the nonlinearity arising from the Guldberg-Waage law. According to our best knowledge, the analytical solution for our system does not exist. Our model allows the calculation of the free and bound-drug protein concentrations at any time point and at any dose after single intravenous bolus dose administration. In order to compare the empirical with simulated data, a numerical approach has been proposed. On the basis of published experimental data the model validation has been carried out. The goodness of fit was satisfactory ($R^2 = 0.99$) and the experimental and simulated AUC (area under the curve) values, as the measure of the bioavailability of drug, were similar (150 M/hxh^{-1}). The preliminary assessment of the model credibility was positive and encouraged further studies.

Keywords: evolution equations, non-linear model, drug protein binding.

1. Linear models in compartmental pharmacokinetics

Pharmacokinetics describes the fate of the drug in the living organism. Together with pharmacodynamics, which deals with a description of the mechanism of drug effects, it belongs to the area of interest pharmacometrics. The basic pharmacokinetic processes that occur after a single intravenous injection of the drug include: a) reversible formation of drug-protein binding, b) distribution of the unbound drug with protein from blood to other tissues, c) elimination of the free form of the drug from the blood via the liver and/or kidneys. In a specific relationship of these processes, the pharmacodynamic effect of the drug appears as a result of interaction between the drug and its specific receptor. Only the free form of the drug, due to the particle's size, has the ability to pass through biological membrane [12]. This allows it to move around in the receptor. It should be noted that each of the above listed processes occurs in a different timescale. "Compartment" assumes that the body consists of a number of compartments, which are homogeneous spaces. In these compartments a drug previously given intravenously is changing its concentration over time. This change of concentration is determined by the processes described above. The essential tenets of compartmental pharmacokinetics are: a constant volume of the compartment and the elimination stream of the drug in proportion to its weight [3]. In one of the variants of the linear two-compartment model

(Fig. 1) there is a central compartment represented by blood in the vascular bed and a peripheral compartment represented by peripheral issues (the body organs).

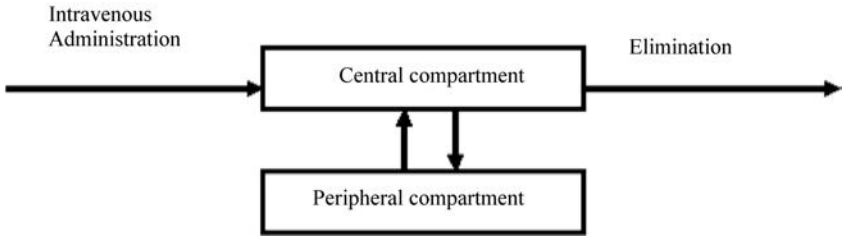

Fig. 1. The linear two-compartment model of a single intravenous administration of drug.

It is assumed that the two-way process of the free form of the drug between compartments and the process of elimination of the drug from the central compartment take place in accordance with first-order reaction kinetics. Variability in concentrations of the drug over time in the central compartment is most often described by the biexponential formula:

$$c(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t}. \tag{1}$$

A_1, A_2, k_1, and k_2 are obtained from the intercepts and slopes of the plasma concentration *versus* time curve by means of nonlinear regression analysis. Thus, A_1 is the Y-intercept of the distribution phase (with a slope, k_1) and A_2 is the Y-intercept of the elimination phase (with a slope, k_1) [4]. Experimentally determined parameters allow predicting the concentration of the total drug at any time. Currently, in compartment pharmacokinetics, the linear models dominate and differential equations for these models have an explicit solution [6].

2. Evolution equations

In the history of the nonlinear equations describing the binding of the drug with plasma proteins the fundamental works appeared in the 70's of the last century [9]. In most of them there are various assumptions describing models and simulations of the concentrations of the drug. Berezhkovskiy published significant work of drug binding kinetics of protein [1, 2]. Presented equations describe the drug concentrations profile vs. time in nonlinear two-compartmental pharmacokinetic model. The model scheme is shown in Fig. 2. The variables in our model are: free drug concentration $s(t)$ in a central compartment, bound drug concentration in a central compartment $c(t)$ and free drug concentration in a peripheral compartment $n(t)$ and drug protein concentration $s(t)$, all depending on time. The parameters are: volume of distribution, association rate constant (k_{on}), dissociation rate constant (k_{off}), distribution rate constant (β), elimination rate constant (α).

The system of evolution equations for a single intravenous administration of hypothetical drug for two-compartmental model has the following form:

$$\frac{\delta s(t)}{\delta t} = -k_{on} e(t) s(t) + k_{off}\, c(t) - \alpha s(t) + \frac{\beta}{V_0}[n(t) - s(t)], \tag{2}$$

$$\frac{\delta e(t)}{\delta t} = -k_{on} e(t) s(t) + k_{off}\, c(t), \tag{3}$$

$$\frac{\delta c(t)}{\delta t} = k_{on} e(t) s(t) - k_{off}\, c(t), \tag{4}$$

$$\frac{\delta c(t)}{\delta t} = V_{eff}\, n(t) = -\beta[n(t) - s(t)], \tag{5}$$

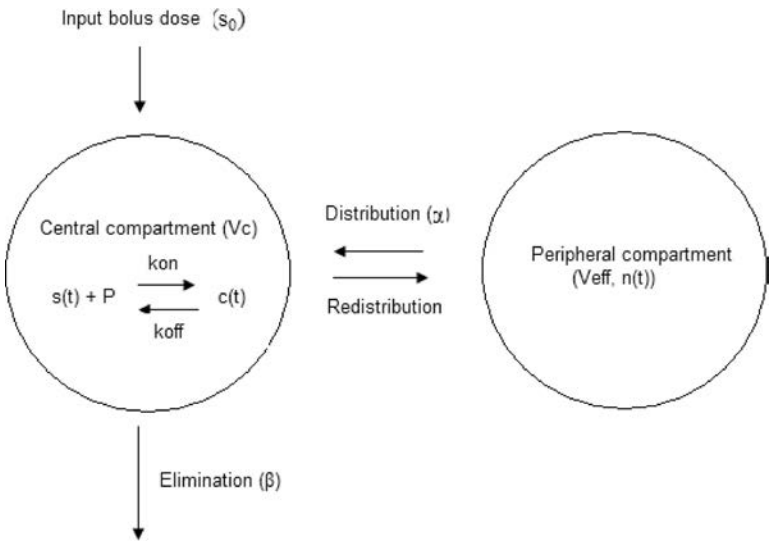

Fig. 2. Two-compartment non-linear model taking into account a drug protein binding.

where V_0 denotes the volume of the central compartment, V_{eff} denotes the volume of distribution (that is, the volume of peripheral compartment), $n(t)$ is the concentration of a free form of a drug in peripheral compartment. After comparing (3) and (4) one can see that the sum

$$c(t) + e(t) \tag{6}$$

is a constant of motion which shall be denoted as Λ:

$$\Lambda = c(t) + e(t) \geq 0. \tag{7}$$

After insertion

$$e(t) = \Lambda - c(t) \tag{8}$$

into (2)–(5) one arrives at

$$\frac{\delta s(t)}{\delta t} = -k_{on}\left[\Lambda - c(t)\right] s(t) + k_{off}\, c(t) - \alpha s(t) + \frac{\beta}{V_0}\left[n(t) - s(t)\right], \tag{9}$$

$$\frac{\delta}{\delta t}\left[\Lambda - c(t)\right] = -k_{on}\left[\Lambda - c(t)s(t) + k_{off}\, c(t)\right], \tag{10}$$

$$\frac{\delta c(t)}{\delta t} = k_{on}\left[\Lambda - c(t)\right] s(t) - k_{off}\, c(t), \tag{11}$$

$$\frac{\delta}{\delta t} V_{eff}\, n\,(t) = -\beta\left[n(t) - s(t)\right]. \tag{12}$$

What is equivalent to:

$$\frac{\delta s(t)}{\delta t} = -k_{on}\left[\Lambda - c(t)\right] s(t) + k_{off}\, c(t) - \alpha s(t) + \frac{\beta}{V_0}\left[n(t) - s(t)\right], \tag{13}$$

$$-\frac{\delta}{\delta t} c(t) = -k_{on}\left[\Lambda - c(t)\right] s(t) + k_{off}\, c(t), \tag{14}$$

$$\frac{\delta c(t)}{\delta t} = k_{on}\left[\Lambda - c(t)\right] s(t) - k_{off}\, c(t), \tag{15}$$

$$\frac{\delta}{\delta t} V_{eff}\, n(t) = -\beta\left[n(t) - s(t)\right]. \tag{16}$$

Since Eqs. (13) and (14) are dependent (they can be transformed one into another after multiplication with -1), we are left with the following system of the tree equations:

$$\frac{\delta s(t)}{\delta t} = -k_{on}\left[\Lambda - c(t)\right] s(t) + k_{off}\, c(t) - \alpha s(t) + \frac{\beta}{V_0}\left[n(t) - s(t)\right], \tag{17}$$

$$\frac{\delta c(t)}{\delta t} = k_{on}\left[\Lambda - c(t)\right] s(t) - k_{off}\, c(t), \tag{18}$$

$$\frac{\delta}{\delta t} V_{eff}\, n(t) = -\beta\left[n(t) - s(t)\right]. \tag{19}$$

The system (16), (17) and (18) relates three dynamical equations and depends on the value of the constant of motion Λ.

In general, it allows defferent initial conditions but for a single intravenous administration of drug at the time $t = 0$ the corresponding initial condition reads:

$$s(t=0) = s_0 > 0, \tag{20}$$

$$c(t=0) = 0, \tag{21}$$

$$n(t=0) = 0. \tag{22}$$

If a drug does not bind with plasma protein then $k_{on} = 0$ and $k_{off} = 0$. For a drug which bounds with plasma protein, $\Lambda > 0$. This model allows the simulation of free and plasma protein-bound drug concentrations after single-bolus intravenous administration of drug (Fig. 2).

To solve the equations described using the numeric method due to the lack of analytical solutions.

3. Parameters of the simulation model and the preliminary process of validation

Recently, Sargent defined the validation process as substantiation that a model within its domain of applicability possesses a satisfactory range of accuracy consistent with the intended application of the model [12].

In order to validate the simulation model we used the results of an experimental study published by Perucca et al. [11]. In this study, 800 mg of valproic acid (VPA) was administered intravenously to six healthy volunteers. The concentration of total drug in blood serum was measured in the following time sequence: 0 h, 0.5 h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 11 h, 14 h, 24 h, 28 h, 32 h, 36 h, 48 h.

The original simulation model parameters were: elimination rate α – 0.06 h^{-1}; distribution rate β – 15 h^{-1}; concentration of protein binding (albumins) – 0.006 M/L; vascular bed volume V_0 – 5 L; distribution volume V_{eff} – 10 L; association rate constant K_{on} – 1; dissociation rate constant K_{off} – 0.001; drug dose s_0 – 0.006 M. The secondary parameters of the simulation model were: AUC_s, AUC_c, and AUC_n. AUC_s is the area under a plot of the free drug plasma concentration versus time curve, AUC_c – the area under a plot of the concentration of drug-protein complex in plasma versus time curve and AUC_n – the area under a plot of the free drug concentration in tissue versus time curve.

Figure 3 presents simulated and experimental total concentrations of the drug.

Figure 4 presents simulated plasma concentration of free form of drug and protein drug bound in central (vascular bed) and peripheral (tissue) compartments.

By means of the least squares method, the mono-exponential, polynomial and bi-exponential curves were fitted to the distribution of experimental and simulated plasma concentration values of VPA. The null hypothesis that the simpler model (the one with fewer parameters) was correct, was estimated by the extra sum of squares F test at the confidence level $P = 0.05$. On the basis of the results of the F test, the best fit to experimental and simulated data proved to be a biexponential

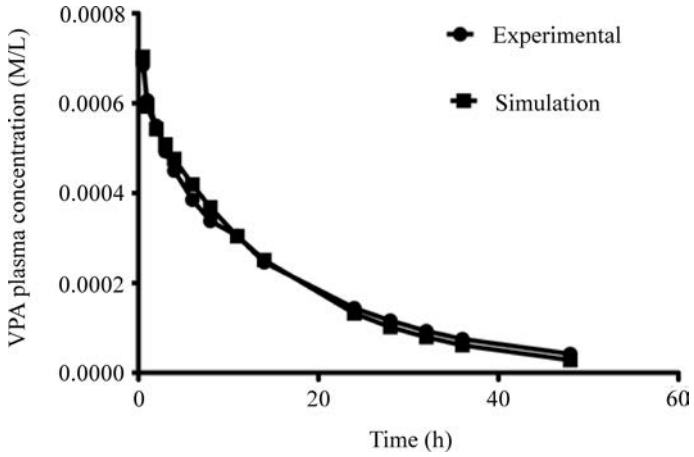

Fig. 3. Simulated and experimental values of the total plasma concentration of the VPA after single intravenous administration of drug (250 mg).

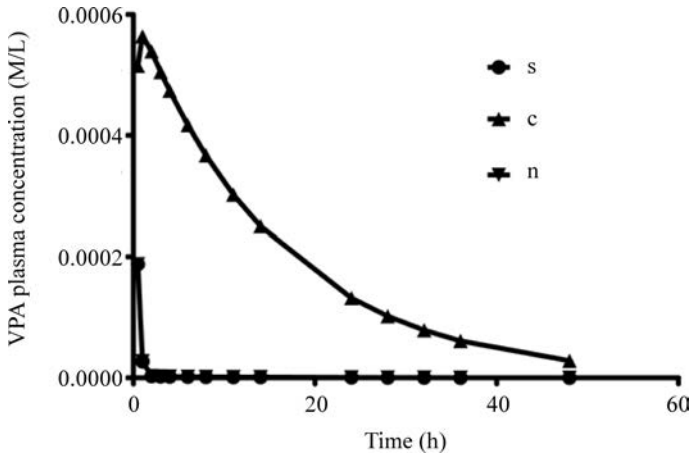

Fig. 4. Simulated values of free (s), albumin bound drug in central compartment (c) and free form of drug in peripheral compartment (n).

function. In order to assess the quality of curve-fitting, we took statistical estimates of the parameters of this function and applied the method of least squares for both empirical and simulated data. Estimation of parameters is carried out using the GraphPad Prism®. The following is a comparison of the curve fitting characteristics. Tables 1 and 2 summarize the best fit values of the

Table 1. The value of parameters A_1 and A_2 for biexponential function fitted to experimental and simulated data; SEM-standard error of mean, CL-confidence limit.

VPA plasma conc.	Best fit-values							
	A_1	SEM	L95%CL	U95%CL	A_2	SEM	L95%CL	U95%CL
Experimental	0.0005	1.56E-05	0.0005	0.0006	0.0002	2.19E-05	0.000162	0.000261
Simulated	0.0006	2.50E-07	0.0006	0.0006	0.0008	1.57E-05	0.000748	0.000819

Table 2. The value of parameters A_1 and A_2 for biexponential function fitted to experimental and simulated data; SEM-standard error of mean, CL-confidence limit.

VPA plasma conc.	Best fit-values							
	k1	SEM	L95%CL	U95%CL	k2	SEM	L95%CL	U95%CL
Experimental	0.0553	0.006334	0.04097	0.06963	0.606	0.1394	0.2906	0.9212
Simulated	0.0643	9.60E-05	0.06408	0.06451	3.989	0.04148	3.895	4.083

biexponential function parameters for experimental data and simulated data (Eq. (1)). Confidence intervals were computed from the standard errors of the parameters.

As shown in Table 1 and Table 2 the model parameters for experimental and simulated data were narrowly within 95% confidence limits. It means that both experimental and simulated data define very well the model parameters.

Table 3 shows R^2 values calculated from the sum of the squares of the distances of the points from the best-fit curves, i.e., two-exponential curve determined by nonlinear regression for experimental and simulated data. The high R^2 means that all the values lie exactly on a straight line with no scatter. It quantifies goodness of fit. This observation is supported by very low values of the standard deviations of the residuals (Sy.x).

Table 3. Goodness of fit quantified by means of R^2 and standard deviation of the rest residuals.

VPA plasma conc.	Goodness of Fit			
	Degrees of Freedom	R square	Absolute Sum of Squares	Sy.x
Experimental	9	0.9989	6.47E-10	8.48E-06
Simulated	9	1	5.25E-13	2.41E-07

Table 4 shows the results of the normality test for two-exponential functions fitted to experimental and simulated plasma concentrations of VPA after bolus intravenous administration of drug.

Table 4. The results of the normality test for two exponential functions fitted to experimental and simulated plasma concentrations of VPA.

VPA plasma conc.	Normality of Residuals			
	D'Agostino & Pearson	P value	Shapiro-Wilk W	P value
Experimental	1.967	0.374	0.9633	0.7774
Simulated	1.45	0.4844	0.9287	0.2924

The results show that the residuals passed the normality test ($P > 0.05$) and all scatter around the two-exponential curve for experimental and simulated data follow a Gaussian distribution.

Table 5 shows the results of the runs test. P values higher than 0.05 means that two exponential curves fitted and described very well both experimental and simulated data.

Table 5. The results of the runs test.

VPA plasma conc.	Runs test			
	Points above curve	Points below curve	Number of runs	P value (runs test)
Experimental	6	8	7	0.4126
Simulated	8	6	10	0.9371

The dependency value for each parameter quantifies the degree to which that parameter is intertwined with the other. In our case, dependence for all parameters is lower than 0.99 (Table 6).

Table 6. The results of dependency analysis.

VPA plasma conc.	Dependency			
	A_1	k_1	A_2	k_2
Experimental	0.9471	0.9777	0.8446	0.9301
Simulated	0.8201	0.9178	0.9874	0.9889

It means that after changing the value of one parameter, one can change the values of other parameters to reconstruct exactly the same two-exponential curves, i.e., the curves fitted for experimental and simulated data. The results of the evaluation of biexponential function to distribution of the concentrations of VPA established experimentally and those calculated numerically are comparable. It can therefore be said that the biexponential function is similarly an accurate fit to the experimental data and to data computed numerically. This legitimizes the conclusion that numerically calculated data values do not differ significantly from the experimental data, and are a good approximation.

4. Preliminary assessment of the model credibility

According to Sargent's definition, model credibility is concerned with developing, in (potential) users, the confidence they require in order to use a model, and in the information derived from that model [12]. Below are three examples of practical use of our simulation model. They can be treated as a preliminary assessment of the credibility of the model.

The calculation algorithm solving differential equations of the system described above allows one to simulate drug concentrations in the vascular bed (central compartment), at any time after single intravenous injection of the drug in any dosage. Especially important is the concentration of the free form of the drug, because only such a form of medicine determines the intensity of the pharmacodynamic effect, toxicity or lack thereof. In addition, the algorithm calculates the AUC_s, AUC_c, and AUC_n, as described above. AUC is one of the key parameters of non-compartmental pharmacokinetics. Contrary to common use of the numerically calculated AUC of the total concentration of drug vs. time curve, our algorithm computes AUC for free (AUC_s) and plasma protein bound concentration of drug (AUC_c) versus time. AUC quantifies the bioavailability of drug, i.e., the amount of the drug available in the vascular bed after its administration. The AUC is an important parameter in the comparison of the same drugs from two different producers. Such a comparing of two drugs is called bioequivalence study [5, 8]. The other application field of AUC calculation is toxicokinetics [14]. It can be used as a measure of drug exposure and answers the query of how long a drug stays in a body. To assess the efficacy of some anti-infective drugs one can use AUC/MIC (minimum inhibitory concentration) that is a pharmacokinetic-pharmacodynamic parameter [10]. Some examples of practical applications of our model simulation are listed below. They relate to the problems and practical aspects associated with variation of the free concentration of the drug in plasma.

Example 1

With the assumption of a linear compartmental model, it follows that multiple injection doses should result in a proportionate increase in the concentration of the drug in the vascular bed and in peripheral tissues. In linear compartmental model pharmacokinetics is assumed to be a linear relationship between the concentration of the drug and his AUC. Our simulation model answers the question of how concentration of drug forms (AUC) will change as a result of the multiplication of their dosage. As shown in Fig. 5, a 10-fold increase in the dose of the drug (VPA) causes a 10-fold linear increase in only AUC of free form (AUC_s).

In the case of the concentrations of other forms of the drug (bound and total), a dose with AUC relationships is non-linear. In some situations, it may be a source of interpretative error.

Example 2

The relationship between the drug and protein binding in the central compartment as described in many publications is mostly devoid of a time constant [4]. Simulation allows to predict concentration

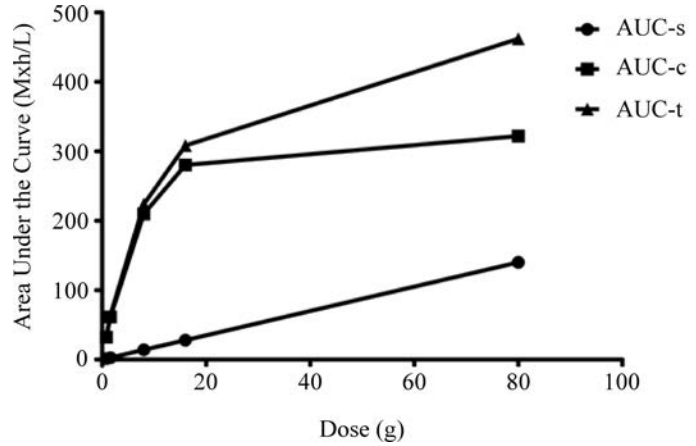

Fig. 5. Analysis of the relationship between dose and the area under the curve for three forms of VPA presented in central compartment (vascular bed): the free (AUC_s), the albumin bound drug (AUC_c) and the total form of drug (AUC_t).

profiles at any time for free and protein-related form of the drug given for the different values of the association constant. An example of simulated variability in concentrations of serum VPA in the central compartment (vascular bed) after its single intravenous injection at a dose of 250 mg, in relation to the association constant, is shown in Fig. 6.

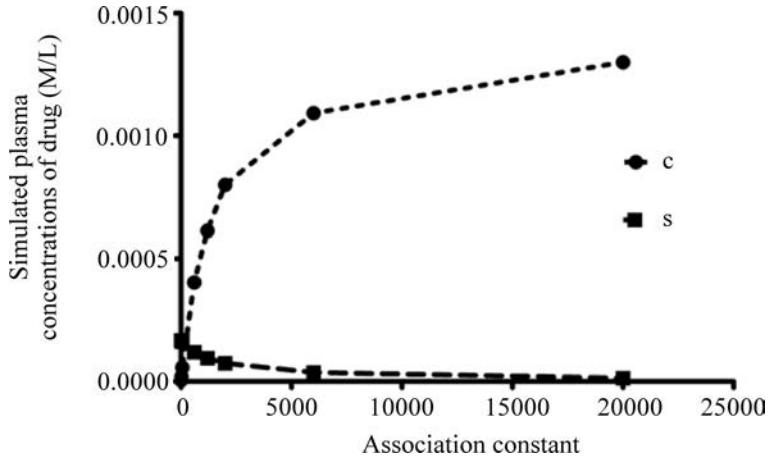

Fig. 6. Simulated plasma concentrations of VPA in the central compartment (vascular bed) after its single intravenous administration of drug in relation to the association constant of drug: s – the free form of drug, c – the bound drug to albumin.

The variability of the association constant rate of the drug to a certain extent, which may occur in the process of pharmacokinetic interaction, may result in a substantial change in the concentration of free form, and may change the effect of the drug.

Example 3

Gugler and Mueller [7] compared concentrations of free form of VPA in healthy volunteers and patients with renal failure. In a population of patients with kidney failure the concentration of free form of VPA in serum was increased and then compared with a population of healthy volunteers [7]. It was correlated strongly with creatinine, while poorly with concentrations of albumin, a protein binding VPA. Simulation of the relationship of the area under the curve for three forms of VPA and albumin concentration in plasma has described this in quantitative terms (Fig. 7).

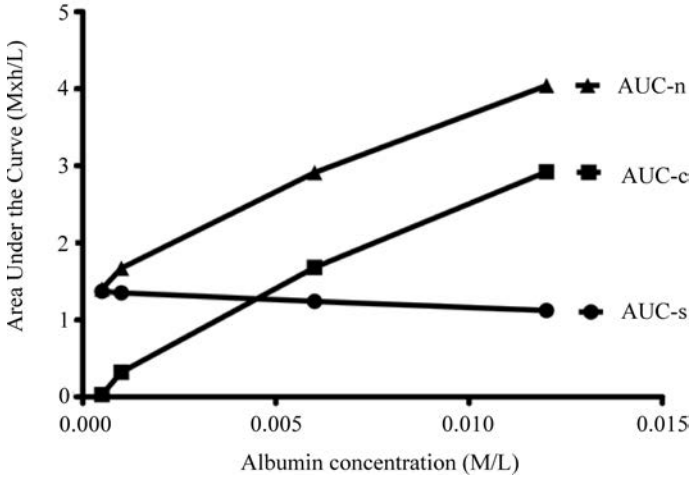

Fig. 7. Simulation of the relations between the area under the curve for two-plasma concentration time – profiles of VPA and the plasma concentration of albumin. Area under the curve time concentration for free form of drug (AUC_s), bound drug to albumin (AUC_c) and free form of drug in peripheral compartment (AUC_n).

The simulation showed that following more than 10-fold reduction in the concentration of albumin in the vascular bed leads to a small (about 10%) increase in the availability of the free form of the drug (AUC_s), and to almost 60-fold reduction in the availability of the form associated with a protein (AUC_c). Simulation confirmed the observation of clinicians that even extreme reduction of the concentration of albumin in the vascular bed will not increase the concentration of free form of a drug, and thus will not cause the severe pharmacodynamic effect. In conclusion, it should be noted that the initial assessment of the credibility of the proposed model has provided interesting results and is an incentive for further research.

5. Conclusion

This work proposed the system of conservation laws as applied to drug concentration profiles vs. time, after a single intravenous administration. Most other models differ in that they simultaneously consider only the sum of the a free and a protein bound form of a drug. Simulation of free-form of the drug is particularly important because of its direct impact on the pharmacodynamic effect of the drug. Application of the Guldberg-Waage law to describe the association and dissociation of the complex drug-protein (albumin) introduces the non-linearity to conservation laws. It is worth mentioning that most of the pharmacokinetic models are linear. According to our knowledge there is no analytic solution for our equations. To compare the empirical data with simulated data this approach required numerical methods. Empirical data were concentrations of VPA measured in the serum of volunteers after administration of single intravenous bolus dose. Satisfactory correlation existed between experimental and simulated versions of total concentrations of the drug and was comparable with the AUC. Preliminary simulation adds to pharmacokinetic praxis and suggests good credibility of the model. It also provides incentive to further study, evaluation and verification of the model. Numerical calculation of the AUC_s and AUC_c, by using the simulation model, can find application in biopharmacy (absolute bioavailability related to saturable transporters or metabolic enzymes), personalized therapy (optimal sampling time) and in toxicokinetics (prediction of drug dose in poisoning).

References

[1] L.M. Berezhkovskiy. Some features of the kinetics and equilibrium of drug binding to plasma proteins. *Expert Opin Drug Metab Toxicol*, **4**: 1479–1498, 2008.

[2] L.M. Berezhkovskiy. Determination of hepatic clearance with the account of drug-protein binding kinetics. *J. Pharm. Sci.*, **101**(10): 3936–45, 2012.
[3] S. Bielawski. *Pharmacokinetic models* [in Polish]. WKiŁ, Warsaw, 1987.
[4] T. Bohnert, L.S. Gan. Plasma protein binding: from discovery to development. *J. Pharm. Sci.*, **102**(9): 2953–2994, 2013.
[5] M. Dickins, H. Waterbeemd. Simulation models for drug disposition and drug interactions. *Biosilico*, **2**(1): 38–45, 2004.
[6] J. Gabrielsson, D. Weiner. *Pharmacokinetic and pharmacodynamic data analysis: concepts and applications.* Swedish Pharmaceutical Society, Stockholm, Sweden, 328–444, 2000.
[7] R. Gugler, G. Mueller. Plasma protein binding of valproic acid in healthy subjects and in patients with renal disease. *Br. J. Clin. Pharmacol*, **5**(5): 441–446, 1978.
[8] R. Lobenberg, G.L. Amidon. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. *Eur. J. Pharm. Biopharm*, **50**(1): 3–12, 2000.
[9] P.J. McNamara, G. Levy, M. Gibaldi. Effect of plasma protein and tissue binding on the time course of drug concentration in plasma. *J. Biopharm. Biopharmaceutics*, **7**(2), 1979.
[10] JW. Mouton, MN. Dudley, O. Cars. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. *J. Antimicrob Chemother*, **55**(5): 601–607, 2005.
[11] E. Perucca, G. Gatti, G.M. Frigo, A. Crema. Pharmacokinetics of valproic acid after oral and intravenous administration. *Br. J. Clin. Pharmac.*, **5**: 313–318, 1978.
[12] R.G. Sargent. Verification and validation of simulation models. *Journal of Simulation*, **7**: 12–24, 2013.
[13] D. Smith, L. Di, E. Kerns. The effect of plasma protein binding on *in vivo* efficacy: misconceptions in drug discovery. *Nat Rev Drug Discov*, **9**(9): 929–939, 2010.
[14] M.J. Wolfsegger, T. Jaki. Assessing systemic drug exposure in repeated dose toxicity studies in the case of complete and incomplete sampling, *Biometrical Journal*, **51**(6): 1017–1029, 2009.