Usability Experience in the Calculation of Pharmacokinetic Parameters With Software: Phenytoin Case

Stefhano Olivo Valdivia[1][0000-0002-2904-8134], Rita Nieto Montesinos[1] [0000-0002-5671-4593], Karim Vera López[1] [0000-0002-7908-8230], Gonzalo Dávila-del-Carpio [1] [0000-0001-7842-9286]

[1] Grupo de Investigación en Neurociencia de la Facultad de Ciencias Farmacéuticas, Bioquímicas y Biotecnológicas de la Universidad Católica de Santa María, Arequipa, Perú.
{2015222641, rnieto, kvera, gdavilad}@ucsm.edu.pe

ABSTRACT: The objective of this research was to evaluate how the use of mathematical models that simulate the drug´s pharmacokinetic, can influence the calculation of pharmacokinetic parameters, being the human-computer-interaction an useful tool to simplify them. For it, we study the effect of P-gp inhibitors on the pharmacokinetics of the anticonvulsant, phenytoin; we co-administered the drugs to a group of rats and then measured the plasma concentrations of phenytoin, from which we calculated the pharmacokinetic parameters, manually and using the WinNonlin software. P-gp inhibitors caused a change in the pharmacokinetics of phenytoin, moving from the open-one compartment model to the open-two-compartment model, this was demonstrated with a better fit of data (r = 0.9871) in the two compartments model. Since complex mathematical models are necessary, the software will be useful, but it is the pharmacokinetic specialists who evaluate and define the model to be used, which demonstrates the importance of HCI.

KEY WORDS: Pharmacokinetic parameters, HCI, compartmental models, Phenytoin, P-glycoprotein inhibitors, WinNonlin software.

1 Introduction

There are numerous definitions of the term pharmacokinetics (PK), Dost, Wagner and Gibaldi [1, 2] describe pharmacokinetics as "the processes of speed change in drug concentrations in the human or animal body", or "the study of speed change of drug concentrations and their metabolites in biological fluids, tissues and excretions, as well as the pharmacological response and construction of appropriate models for the interpretation of such data" and finally "the study of the temporal evolution of drug levels and their metabolites in the different fluids, tissues and emunctories of the organism and the mathematical relations necessary to develop the appropriate models to interpret such data".

The main objective of pharmacokinetics is to clarify the relationships between the pharmacological or toxicological response and the levels of the drug or its metabolites in different body fluids and offers a way to improve and optimize the individual treatment of patients, being Clinical Pharmacokinetics a fundamental tool in the achievement of this objective and key to establish appropriate dosing regimens [2, 3].

The pharmacokinetic parameters (P.P.) of drugs are unique and specific, depending on the active ingredient (A.I.), and the pharmaceutical dosage form. Thus, each drug is described by its pharmacokinetic parameters such as absorption (Ka) and elimination (K) constants, half-life time ($t_{1/2}$), apparent distribution volume (Vd), systemic clearance (Cls), metabolization constant (km), unchanged drug excretion constant in urine (ke), liver clearance, bioavailability factor (F), among others, or the so-called bioavailability parameters, namely the area under the plasma concentration curve vs. time (AUC), the maximum plasma concentration and maximum time [4 - 7].

If it is considered that each fluid, cell, tissue, organ and system have different physical-chemical characteristics and different degrees of affinity for drugs, the human organism has multiple compartments, in which each of them would act as an individual compartment [3, 4, 6]. Drugs move between compartments and their distribution are complex; these events occur simultaneously, requiring mathematical and statistical models to estimate drug doses and drug's efficacy over the time for a given dose. A model is a hypothesis, which uses mathematical terms to describe quantitative relationships. The predictive capacity of a model is based on the appropriate selection and development of the mathematical function that parameterizes the essential factors governing the kinetic process [7]. Consequently, specialized software which facilitate mathematical modeling for the calculation of P.P. are a very useful tool, where the interaction of the expert in clinical pharmacokinetics with the software, is crucial.

Hefley in 1992 defined the Human-Computer Interaction (HCI) as “the discipline that design, evaluate and implement interactive computer systems for human use; and to the study of the most significant related phenomena” [8].

The difficulty in mathematical modeling of pharmacokinetics and the calculations is relevant to many researchers, for example, Ping Wang et. al. in 2019, developed a TCM-ADMEpred method, which implies a novel strategy for the prediction of pharmacokinetics in traditional Chinese Medicine (TCM*)*; the method was used to predict the AUC of 3 alkaloids in Yuanhu Zhitong (YZP) and 2 coumarins [9].

Another recent study by Zhaomin Dong et. al. presents a web-based application, pkweb (https://pkweb.hhra.net), with an easy-to-use interface and a range of functions for analyzing PK data. Capabilities include PK data simulation, which integrates 27 modules (e.g., intravenous bolus (IV) injection, IV infusion and extravascular administration) and models the suitability of the PK data [10]. Buffington D.E. et al. and Leader W.G. et al., developed pharmacokinetic software programs for clinical decision making, which were designed to assist the physician in the analysis, prediction, interpretation and reporting of serum concentration of several drugs using Bayesian and non-Bayesian methods [11].

Consequently, the use of specialized software nowadays is a fundamental aid for the calculation of P.P.; and it is a clear example of HCI, where the computer programs are specially designed to provide solutions such as [12 - 14]:

1. Adequacy of drug concentration vs. time data to a series of pharmacokinetic models and the choice of the best one in the statistical description of the data.

2. Adequacy of data within a user-defined PK or pharmacodynamic (PD) model
3. Simulation: Since simulation processes involve a numerical solution of the equation with predefined precision.
4. Experimental design: Allowing the description of the curve and the model.
5. Applications in Clinical Pharmacokinetics: some software are available for the clinical monitoring of drugs with a narrow therapeutic index.
6. Computer programs for teaching.

In the teaching-learning methodology, it is very helpful to apply the theories of constructivism and cognitivism. Constructivism is a theory about learning and not a teaching strategy or method. It states that students must actively construct their own knowledge, making them believe and respond to their own hypotheses, increasing the student's confidence in their own critical thinking skills. Students must build their own compartmental and mathematical models, making the transit of drugs in the body fit them. Cognitivism and constructivism can be applied to curricular design and choice of educational methods. David Kolb's theory of experiential learning has many potential applications related to constructivist. In this theory, learners enter a cyclical process of taking part in a concrete experience, reflecting on this experience to formulate an abstract conceptualization of the process, which is then tested through active experimentation [16], which is the nature of pharmacokinetic evaluations.

2 Calculation methodology

The starting point for a pharmacokinetic study, is to define the type of samples to be analyzed; in the animal organism there is access, mainly, to two important fluids in which the distribution of drugs can be investigated: blood and urine. The complexity of the mathematical model designed to find the interrelationship will depend on the number of systems in which it is proposed that the drug is distributed [1, 17]. However, a simple and useful pharmacokinetic tool is based on Compartmental Models; in this analysis based on linear pharmacokinetics, there is a direct proportionality of transfer rates between different compartments and concentrations will depend primarily on the route of administration, we can have the following cases [7, 18]:

1. Intravenous administration, either rapid (bolus) or slow intravenous infusion
2. Extravascular administration (referred to any other route with absorption)

If, for example, we consider an IV administration to the organism and assume a homogeneous distribution throughout the body, we speak of an open one compartment model (MA1C). In pharmacokinetics, it is considered that P.P. are constant for a given A.I. and the Vd and the K can be estimated from plasmatic concentrations vs. time data [7, 17, 18]. It is also important to consider the kinetics that the A.I. follows within the organism; in most cases, it is assumed that the drugs follow first-order kinetics, that is, dependent on the amount of A.I.; furthermore, it is considered a unidirectional sense of the drug's transit, which is why we speak of open models [3, 17]. Within the variants of compartmental analysis (Fig. 1) we have the following possibilities [7, 17]:

1. Open model of one compartment (MA1C), where a homogeneous distribution of A.I. is assumed, with a single central compartment (Fig. 1 - A).
2. Open model of two compartments (MA2C), where the body tissues are classified into two categories: those that are instantly balanced and those that require some time to reach equilibrium (peripheral compartment) (Fig. 1 - B).
3. Multi-compartmental models, where the drug reaches the central compartment, but with a different speed towards other peripheral compartments.

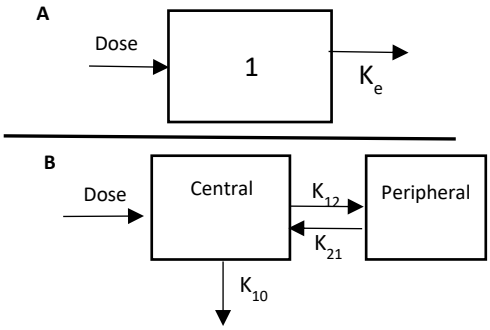

Fig. 1 Compartment Models after IV bolus administration of a drug MA1C (A) and MA2C (B) [20].

It is possible that many drugs may follow non-linear pharmacokinetics and the kinetic process is governed by the magnitude of the dose [1, 7, 17]; other drugs give rise to dose-dependent kinetics and are governed by the Michaelis-Menten equation [1, 7].

Numerous linear differential equations are involved in pharmacokinetic calculations. Although these differential equations are integrated by conventional methods, these often require much time and are impractical; it is common to use the Laplace transformation, a methodology that consists of replacing the independent variable (time) by the Laplace operator [1, 7].

In summary, the calculation of the P.P. must take into account the following considerations [1, 7, 17]:

1. Fluid in which the study will be carried out.
2. To define if the concentrations of the A.I. or the metabolites are quantified.
3. Route of administration of the drug.
4. Type and kinetic order that follows the A.I. contained in the drug.
5. Model of compartments to which the A.I., MA1C, MA2C or others are most suitable.
6. General and specific method to be used for the calculation, based on slopes or mass balance.

Among the most used software programs, we have the following: PCNonlin, WinNonlin, SAS, RSTRIP, PKAnalyst for Windows, DIFFEQ Pharmacokinetics Library, P-STAT, STELLA, NONMEM, MKMODEL, ADAPT II [7, 12 - 14].

In these decisions a series of mathematical equations are usually used, that will later be operated by the software; here we propose some equations solved after the integration of differential equations, and that will serve us as a tool to demonstrate how the decision of the human specialized in clinical pharmacokinetics will define the appropriate use of the software for the calculation of P.P. [1, 3]:

$$Cp_t = \frac{Do}{Vd} e^{-Kt} \quad (1)$$

Equation (1) allows us to calculate pharmacokinetic parameters from plasma concentration data (Cp) after administration of a drug trough IV bolus; the model assumed is an MA1C [1, 5, 7].

$$Cp_t = Ae^{-\alpha t} + Be^{-\beta t}$$
$$A = \frac{Qo\ (\alpha - k_{21})}{Vc\ (\alpha - \beta)} \quad (2, 3)$$

$$B = \frac{Qo\ (k_{21} - \beta)}{Vc\ (\alpha - \beta)} \quad (4)$$

When an MA2C is assumed, the time it takes for the drug to reach equilibrium depends on the degree of affinity for the tissue or tissues that make up the peripheral compartment; if the log of Cp is plotted as a function of time, we obtain a biexponential curve with two clearly defined slopes; this curve is defined by equation (2), where the values of A and B are expressed by the relationships of equations (3) and (4), where Vc is the volume of distribution of the central compartment [1, 7].

$$Cp_t = \frac{koFDo}{Vd(ka - K)}(e^{-Kt} - e^{-Kat}) \quad (5)$$

Equation (5) allows the calculation of the Cp of a drug from Cp data, after its extravascular administration, using the method of the residuals (Feathered) [1 - 3, 6, 7].

$$C_{av}^{\infty} = \frac{1{,}44\, Do\, t_{1/2}\, F}{Vd\ \tau} \quad (6)$$

Equation (6) allows us to calculate the individualized dosage regimen (Do / τ) for a patient, where τ is the dosage interval when the effective plasma concentration (Cav) to be achieved within the therapeutic range is known [1, 2, 9].

3 Results and discussion

The aim of this work was to demonstrate numerically how the error in the definition of the mathematical model to be used, can induce errors in the calculation of the P.P. and determine that wrong dosage regimens are established, especially in risk patients.

Our research group studied the impact of the administration of P-glycoprotein (P-gp) inhibitors, an efflux pump expressed in several organs and barriers of the body, on the pharmacokinetics of the anticonvulsant, phenytoin. The study was carried out on

male Sprague-Dawley rats, weighing between 250 and 280 grams. In the design of the study, three experimental groups were used, one of which was given phenytoin and the other two phenytoin with different doses of P-gp inhibitors.

The pharmacokinetic calculations were made manually and we compared them with the data obtained with the Phoenix WinNonlin Software - Phoenix 64 version 8.1.0.3535, which works with Windows and is used in PK, PD and NCA [21]. For the processing of the obtained experimental data we use the modules shown in Fig. 2.

Fig. 2. Modules of the WinNonlin software used in the computational calculation of P.F. [22].

The results presented are referred to individuals of the group of male Sprague-Dawley rats, to which the anticonvulsant phenytoin was administered by IV, at a dose of 15 mg/kg of weight, plus the P-gp inhibitor, at a dose of 2 mg/kg of weight (Treatment 1), obtaining the following results:

Table 1. Plasma phenytoin concentrations after IV administration of the association phenytoin (15 mg/kg) and P-gp inhibitor (2 mg/kg) in male Sprague-Dawley rats (n = 2).

Treatment 1		
Time (minutes)	Rat-1 (R-1) Cp (µg/mL)	Rat-2 (R-2) Cp (µg/mL)
5	36.13	36.13
15	31.55	31.56
30	19.22	19.22
45	11.68	11.68
60	9.86	9.88
180	2.31	2.62
360	1.36	1.22
480	0.83	1.01
600	0.77	0.82
720	0.71	0.66

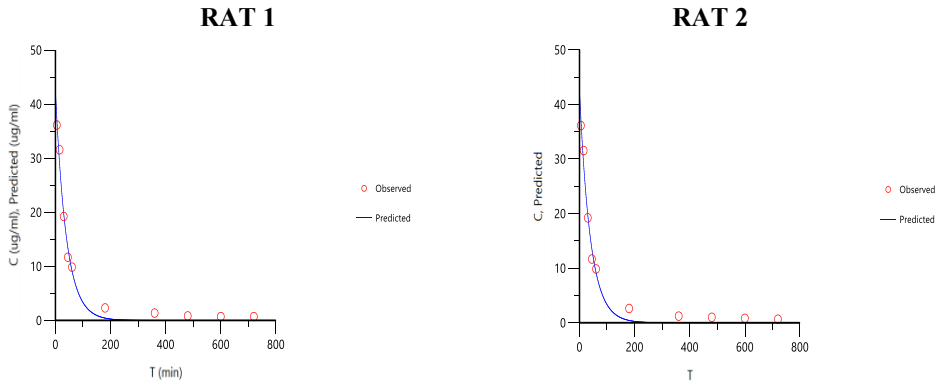

Fig. 3. Plasma concentration profiles vs. time after IV administration of the association phenytoin (15 mg/kg) + P-gp inhibitor (2 mg/kg) in male Sprague-Dawley rats.

The experimental data in Table 1 were used to calculate the P.P., using WinNonlin software; a good fit is appreciated when comparing the observed values, shown in red in the graphs in Fig. 3, with those predicted from the model fit using a MA1C (in blue); furthermore, if we compare the P.P values calculated for the two rats shown in Table 2, we observe no significant statistical differences in terms of AUC, K, $t_{1/2}$, maximum concentration (Cmax), Vd and Cls.

Table 2. Pharmacokinetic parameters of phenytoin administered by IV with P-gp inhibitor in male Sprague-Dawley rats, calculated using WinNonlin software for a MA1C (n = 2)

	R-1			R-2		
Parameter	Estimate	S.E.	C.V. (%)	Estimate	S.E.	C.V. (%)
AUC (ug/ml)*min	1663.38	94.60	5.69	1664.96	98.03	5.89
Ke (min^{-1})	0.0255	0.0021	8.08	0.0255	0.0021	8.37
$t_{1/2}$ (min)	27.14	2.19	8.08	27.19	2.27	8.36
Cmax (ug/ml)	42.47	1.82	4.29	42.45	1.88	4.44
Vd (ml/kg)	353.19	0.0053	4.29	353.36	0.0055	4.44
Cl_s (ml/min/ kg)	9.01	0.0002	5.90	9.01	0.0002	5.89

Calculations with the same data were made manually, using the logarithmic version of equation (1) for a MA1C and first order kinetics, observing that the linear correlation of plasma concentration vs. time in both experimental animals is not very high, with a value of r = 0.9248 for R1 and r = 0.9309 for R2, which leaves some doubts as to whether the kinetics of phenytoin co-administered with the P-gp inhibitor follows a MA1C. The pharmacokinetic parameters calculated manually are shown in table 3, compared with those found with the WINNONLIN software.

We can appreciate similarity in the P.P. calculated for the two experimental animals by the same method, but between methods there are very significant statistical differences, which demonstrates that the MA1C model used is not the most adequate and that the software only processes the mathematical calculations indicated, while it is the

specialist in pharmacokinetics who observe, analyze and evaluate the situation in order to choose the best model, as is deduced from the work of Zhaomin Dong et. al [10].

Table 3. Pharmacokinetic parameters of phenytoin administered by IV with P-gp inhibitor in male Sprague-Dawley rats, calculated manually for a MA1C and compared with WINNONLIN.

	Treatment 1			
Parameter	Manual Rat 1	Manual Rat 2	WINNONLIN R1	WINNONLIN R2
AUC (ug/ml) *min	2868.74	2724.91	1663.38	1664.96
K (min^{-1})	0.0055	0.0055	0.0255	0.0255
$t_{1/2}$ (min)	126.46	126.79	27.14	27.19
Cmax (ug/ml)	17.77	18.06	42.47	42.45
Vd (ml/kg)	846.50	830.67	353.19	353.36
CL_s (ml/min / kg)	4.64	4.54	9.01	9.01

In the correct determination of P.P. using software, it is important to apply computational thinking, which has a high level of abstraction and an algorithmic approach to solve any type of problem, which involves designing systems and understanding human behavior by taking advantage of the fundamental concepts of computing. In the same way, in the teaching-learning process, the theories of constructivism and cognitivism can be applied, where the students themselves are the ones who build knowledge, making them believe and respond to their hypotheses, increasing the student's confidence in their own critical thinking skills [15, 23]; in this case, the phenytoin has a very changeable pharmacokinetics, so when it is administered with the P-gp inhibitor, it is essential to define the mathematical model to which the behavior of phenytoin is adjusted, because it will depend on it whether the calculated P.P. are corrects and whether the adjustment of the dosage regime of this anticonvulsant is appropriate, since if it is not done correctly, the patient's life may be endangered.

There is evidence of the changing pharmacokinetics of phenytoin, so drug resistance in patients with epilepsy is approximately 30%, resulting in refractory epilepsy. The pharmacokinetic hypothesis suggests that antiepileptic drugs, such as phenytoin, do not reach the target tissues in the concentrations required for therapeutic action, resulting from the active efflux of P-gp, a protein expressed in several human tissues and locally over-expressed in the blood-brain barrier (BBB). P-gp confers intrinsic resistance to normal tissues to eliminate unnecessary and toxic exogenous substances, or their metabolites outside the body and is thought to play an important role in the origin of refractory epilepsy. Studies by Ming-Liang Lai et al. suggest that genetic polymorphism of P-gp may affect the efficacy of phenytoin by decreasing absorption or increasing elimination at the central nervous system (CNS) level [24, 25].

There are also antecedents of Michaelian behavior of phenytoin; Aarons Leon and collaborators, determined the maximum metabolic rate (Vm) and the Michaelis-Menten constant (Km) of phenytoin; Zaccara, G. and collaborators carried out a retrospective study in 282 patients with epilepsy to evaluate the predictive performance of

pharmacokinetic methods for individualized dose of phenytoin, using the linearized method of Michaelis-Menten [26, 27].

Consequently, it is clear that the administration of a P-gp inhibitor with phenytoin, makes its pharmacokinetics change, which clearly evidences the need for the expert in pharmacokinetics to interact with the computer, and to be able to define the mathematical models to be used.

When adjusting the average data of the two rats according to a MA2C using the same software, we appreciate an almost perfect adjustment between the observed and predicted data (Fig. 4), obtaining a value of r = 0.9871 in the distribution phase.

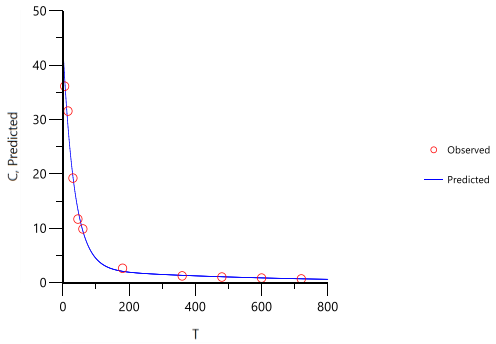

Fig. 4. Plasma concentration profile vs. time of average data, after IV administration of the association phenytoin (15 mg/kg) + P-gp inhibitor (2 mg/kg) in male Sprague-Dawley rats.

We perform the calculations of P.P. for phenytoin following an MA2C, manually (residual method) and using WinNonlin software, with average data, which can be seen in table 4.

Using the MA2C, a greater similarity between the parameters calculated manually and by the WinNonlin software can be clearly seen. This in vivo change in phenytoin pharmacokinetics is due to the joint administration of the P-gp inhibitor, which blocks the action of the P-gp as an efflux pump at the CNS level.

In order to realize the magnitude of the error that can be induced by the use of an erroneous mathematical model, we calculated the dosage regime of phenytoin as a function of the maximum Cp and the average Cp (equation 6), using the P.P. calculated for an MA1C, obtaining a Do = 7.06 mg/kg every 8 hours and with those obtained for an MA2C, which has a better adjustment, the Do = 31.14 mg/kg every 8 hours. The role of usability and the theories of constructivism and cognitivism can be applied, however, in order for these new technologies to be more effective; the contributions from the field of HCI will be critical. [28].

Table 4. Pharmacokinetic parameters of phenytoin administered by IV with P-gp inhibitor in male Sprague-Dawley rats, calculated manually and using WinNonlin software (MA2C).

	Rx	
Parameter	Estimate with WinNonlin	Estimate Manually
AUC (ug/ml) *min	2802.0263	3013.61
A (intercepto)	40.49	42.37
B (intercepto)	2.55	3.09
α (min^{-1})	0.0288	0.0257
β (min^{-1})	0.0018	0.0023
K (min^{-1})	0.0154	0.0151
$t_{1/2}$ (min)	45.12	45.93
$t_{1/2\ \alpha}$ (min)	24.08	26.92
$t_{1/2\ \beta}$ (min)	378.92	306.32
Cmax (ug/ml)	43.04	45.47
K_{12} (min^{-1})	0.0118	0.0091
K_{21}(min^{-1})	0.0034	0.0039
Vc (ml/kg)	348.51	329.92
V_{ss} (ml/kg)	1558.04	1104.04
V_b (ml/kg)	2981.70	2200.12

Index:

α: distribution phase speed constant
β: disposition phase speed constant
$t_{1/2}$ **α:** average distribution half life time
$t_{1/2}$ **β:** average disposal half life time
A: distribution phase intercept
B: phase disposition intercept
Cmax: maximum plasma concentration
Vb: volume of distribution in the body
Vss: volume of distribution in the steady state
Vc: apparent volume of distribution of the central compartment
K12: transfer speed constant from central to peripheral compartment
K21: transfer speed constant from peripheral to central compartment

Thus, in the study of pharmacokinetics, a software is an important tool to help in many of the theoretical and practical aspects. The aim of this article is to provide a computational approach in the field of pharmacokinetics, making it clear that a software only processes information mechanically, the one who decides which pharmacokinetic and mathematical model to use is the expert in clinical pharmacokinetics, so the HCI is a very important tool to help.

4 Conclusions

The concomitant administration of phenytoin with P-gp inhibitors causes significant changes on phenytoin pharmacokinetics as a result of the inhibition of the active efflux of P-gp. This leads to vatiations on the calculated pharmacokinetic parameters of phenytoin depending on the compartment model used. In this study it was demonstrated that the adjustment of the data to an MA1C is relatively good, with an average value of r = 0.9272, which could lead to calculation errors, but when the MA2C was applied,

the data adjusted much better, with a value of r = 0.9871 in the distribution phase. This is why the differences in the P.P. calculated manually and using WinNonlin software show significant differences for an MA1C, while there are no significant differences when the correct model, the MA2C, is used. This allows us to conclude that the usability of specialized software facilitates the calculation of the P.P., however, it is the expert in pharmacokinetics who must decide which model to use, demonstrating thus the importance of human-computer interaction.

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