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PHARMACOKINETICS OF A SINGLE DOSE OF DIGOXIN IN HEALTHY VOLUNTEERS USING THE LINUX OPERATING SYSTEM

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ABSTRACT

Knowledge of the pharmacokinetics (PK) of digoxin is essential in optimizing the tolerability and efficacy of this drug. There are several commercially available software packages available to calculate PK parameters, but many are costly to purchase and maintain. For this reason, they have become inaccessible for many pharmacology students, mainly in developed countries. The aims of this study were to study the PK of digoxin in the Open Office program in Linux to calculate the PK parameters, and to compare the results of this program with two different software packages, Pharmkit and Trapule. 24 healthy volunteers (both sexes) received a single oral dose of 0.5 mg digoxin (2 tablets containing 0.25 mg), in an open and randomized study. Good precision and accuracy values were obtained, shown by the low percent values clearly below 14.08%, at the three concentration levels studied for precision, and the accuracy of the assay was within ±10.99% of the actual value for digoxin. There was no significant difference between the means of all calculated PK parameters with 95% confidence interval. The digoxin PK parameters observed in our study are

in accordance with those found in the literature in healthy volunteers for a single 0.5 mg oral (tablet) dose. Our results conclude that the PK parameters can be calculated by OpenOffice calc, developed in the Linux operating system.

Keywords: Digoxin, Pharmokonetic, Linux system, Pharmkit Program, Trapule Program

INTRODUCTION

Digoxin is the most commonly prescribed cardiotonic glycoside worldwide. It is used in the treatment of congestive heart failure and atrial arrhythmias. It is also believed to be beneficial in pulmonary arterial hypertension as a support treatment **[1, 2]**, and can also reduce the hospitalization rate **[3]**. The main problems with digoxin therapy are its narrow therapeutic index and life-threatening toxicity when an overdose is administered. Monitoring of plasma digoxin concentrations is necessary for safe and effective therapy, and is also helpful in the evaluation of the patient`s treatment compliance **[4]**. There are different reports about the appropriate therapeutic range for digoxin. Some authors suggest the range of 0.8-2.0 ng/mL **[5-8]**, while others believe that the optimal serum digoxin concentration is between 0.5-1.5 ng/mL **[9-11]** and 0.5-2.0 ng/mL **[12]**. The maximum tolerable concentration is usually around 2.0 ng/mL. However, the optimal therapeutic concentration of digoxin shows great inter-individual variation, as does the toxic concentration. Changes in this concentration results in pharmacokinetic (PK) interactions with other drugs, leading either to a clinically significant reduction of the pharmacologic response or to an increase in the incidence of unwanted toxic effects.

Knowledge of the PKs of digoxin is essential in optimizing the tolerability and efficacy of this drug **[12, 13]**. The variability in digoxin disposition creates a difficulty in the choice of the correct dosage by the physician. For a given daily dosage, steady-state serum digoxin concentrations vary greatly from patient to patient **[14]**. Several studies have shown considerable inter-individual differences in bioavailability **[15-17]**. Because of this large interpatient variability, an appropriate dosage regimen should be developed for individual patients. To avoid toxicity and retain efficacy, concurrent use of several digoxin brands requires the bioequivalence and interchangeability of the preparations used **[18-20]**.

While the teaching of PKs is acknowledged to be a key aspect of the core curriculum in clinical pharmacology and therapeutics, it is also widely acknowledged to be a very difficult part of the curriculum to teach. Medical and pharmaceutical students often find it difficult to conceptualize PKs. Various software programs demonstrating experimental PK are being used to teach pharmacokinetics. These softwares

help in reinforcing theoretical knowledge of different drugs acting on various systems in the body. There are several commercially available software packages to calculate PK parameters, but that are costly to purchase and maintain. For this reason, they have become inaccessible to many pharmacology students, mainly in developed countries. In order to improve accessibility for researchers, students and educators, we have employed a free program to determine PK parameters, developed in Linux. The development of this program will be very useful in countries where computers are now sold in stores equipped with the Linux operating system, as in Brazil. Linux is a Unix-like computer operating system assembled under the model of free and open-source software development and distribution. The aims of this study were to develop the program in OpenOffice in Linux, to calculate the digoxin PK parameters, and to compare the results of this program with two different software packages, Pharmkit employed by many authors, such as, Stuart-Harris et al. **[21]**, and Patani et al. **[22]**, and Trapule developed by Wijnand **[23]**.

MATERIALS AND METHODS

Chemicals and Reagents

The immunoassay for digoxin was obtained as a reagent kit (AxSYM Digoxin II lot number 87177Q100 and 89729Q100) from Abbott Laboratories USA and used in accordance with the manufacturer's stated protocol. Digoxin® (lot number 2H7557F) was obtained from Glaxo Wellcome S.A. Laboratory, Brazil. Methanol (HPLC-grade) was purchased from Merck, and the digoxin reference standard from Sigma (lot number 78H8809). Blank human blood was collected from healthy, drug-free volunteers. Plasma was obtained by centrifugation of blood treated with K3-EDTA. Pooled plasma was prepared and stored at approximately -20ºC until needed.

Human Subjects

Twenty-six male volunteers were enlisted. They were required to be aged between 18 and 65 years, in good physical and mental health, with body mass index (BMI) of 18.5 to 28 (Dietary Guidelines Advisory Committee, 1995), and any who had a history of relevant drug hypersensitivity, were excluded from the studies.

Study Design

This was a single-center, open-label, randomized, single-sequence study. In the study, subjects fasted for at least 10 hours prior to the administration a single oral dose of 0.5 mg digoxin (2 tablets containing 0.25 mg) from Glaxo Wellcome S.A. Laboratory, Brazil, with 200 mL of tap water at 8:00 a.m. Subjects remained fasting for 4 h after drug administration. Standard meals were served 4 and 8 h after medication. Water was allowed *ad libitum* before dosing and from 1 h after dosing.

Plasma Samples

Blood samples (10 mL) were collected into K3-EDTA tubes through an inserted catheter placed in the upper forearm at 0 h (immediately prior to dosing), and then at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 8, 12, 24, 48, 72, and 96 hours following administration of digoxin. After each sample was taken the cannula was sealed with 1 mL of heparinized saline. For each blood sample withdrawn, the first 2 mL of blood withdrawn was taken into a separate syringe and discarded. The tubes were immediately mixed by gentle inversion to prevent coagulation and then placed on recycled ice prior to centrifugation. Samples were centrifuged at 1500 g for 15 minutes at room temperature 30 minutes or less after collection to separate plasma from cells. Plasma samples were stored at –20 °C until analysis. Subjects were hospitalized during the first 24 h following oral administration of digoxin.

Drug Assay

Plasma digoxin concentrations were measured with MEIA kits using an AxSYM analyzer. The AxSYM Digoxin II® assay uses a solution of latex coated with a digoxin-specific capture molecule. Once this solution and 150 uL patient plasma were combined, the mixture was transferred to a glass filter matrix in which the captured molecule-drug complex was retained. A conjugated solution containing alkaline phosphatase was then combined with 4-methylumbelliferyl phosphate and was also added to the glass fiber matrix. The conjugated solution catalyzed the hydrolysis of 4-methylumbelliferyl phosphate to methylumbelliferone. The measurement of fluorescent methylumbelliferone was proportional to the concentration of digoxin. This assay does not require any pretreatment of samples for protein precipitation. The samples were analyzed with standards and quality control samples. Standard curves were constructed and digoxin concentrations in serum samples were interpolated. Because the linearity of MEIA assay can be extrapolated to zero, any value measured was considered an apparent digoxin value.

Pharmacokinetic Assessments

Non-compartmentalized PK parameters were derived from the digoxin plasma concentration-time profiles. The PK evaluations were performed using OpenOffice software in the Linux platform and were compared to the values obtained by Pharmkit software (Version 2.0), an interactive pharmacokinetic analysis program **[24]** and by Trapule developed by H. P. Wijnand **[23]**. C_{max} and t_{max} values were determined directly from blood plasma concentrations. AUC_t values were calculated by the trapezoidal rule using the following formula: $AUC_t=\Sigma_i\ [(C_i+C_{i-1}/2)x(t_i - t_{i\ -1})]$. The first-order terminal elimination rate constant (k_e) was estimated by linear regression from the points describing the elimination phase in a log-linear plot; from this constant the terminal

elimination half-life was calculated ($t_{1/2}$=ln2/k_e). AUC_t was extrapolated to infinity by adding C_{last}/k_e, where C_{last} denotes the concentration measured at T_{last} to yield AUC_∞ (AUC_∞ = AUC_t + C_t/K_e). The apparent clearance (CL) was calculated as dose divided by AUC_∞ (CL=Dose/AUC_∞), and the apparent volume of distribution (V_d) was calculated as dose divided by the product of AUC_∞ and k_e (Vd=Dose/AUC_∞ . k_e).

Statistical Analysis

Statistical analyses were performed using the Paired *t*-test, two-tailed to compare differences in the means for the PK parameters of digoxin obtained by three methods. We considered a difference statistically not significant at a 95% confidence interval ($p<0.05$).

RESULTS AND DISCUSSION

Validation of the Method

A stock solution of digoxin was prepared in methanol at concentrations of 100 ng/mL. The analysis was carried out for each concentration. The quality control samples were prepared in blank plasma at concentrations of 0.9, 1.9 and 3.2 ng/mL (low, medium and high controls, respectively). The method was validated according to the guidelines of the International Conference on Harmonization (ICH) for the validation of the following parameters: linearity, limits of quantification, and intra- and interday precision **[25]**.

Limit of Quantification (LOQ)

The LOQ defined as the lowest concentration at which both the precision and accuracy are 8.87 and 102.07, respectively, is 0.3 ng/mL.

Linearity

Linearity was determined to assess the performance of the method. A linear least-squares regression analysis was applied to the concentration ratios of digoxin vs. the concentrations of the six plasma standards of digoxin (0.3, 0.5, 1.0, 2.0, 3.0, and 4.0 ng/mL) in triplicate to generate a calibration curve. The method was considered linear for digoxin concentrations, and the calibration curves showed a linear relationship for digoxin. The equation of the line obtained by least squares linear regression analysis is Y=0.9966.x – 0.0356, and the correlation coefficient is 0.9995.

Precision

Values for intra and interday coefficients of variation were determined by replicate analyses (n = 6), either on the same run (intraday) or on six separate days (interday). Three concentration levels were selected for validation (0.9, 1.9, and 3.2 ng/mL). The data on precision are presented in Table I. For digoxin, good precision and accuracy values were obtained, shown by the low percent values clearly below 14.08%, at the three concentration levels studied, and the accuracy of the assay was within ±10.99% of the actual value for digoxin.

Recovery

The recovery test was never done since the samples were not extracted but were processed through enzymatic reactions.

Stability

The stability tests indicated no significant degradation under the conditions described. To assess stability, quality control plasma samples (0.9, and 3.2 ng/mL) were subjected to short-term (6 h) at room temperature, three freeze/thaw (-20ºC) cycles, 6 h autosampler stability (20°C) and a long-term incubation (80 days). After each of these processes, the digoxin concentrations were measured and compared with freshly prepared samples.

Demographics and Baseline Characteristics

Twenty-six healthy volunteers (13 males and 13 females) participated in the study, two subjects discontinued the study drug; 24 completed treatment according to the protocol. Their mean age was 23.42 years (range 18–44 years), their mean weight 62.47 kg (range 51.5–81.0 kg), and mean body mass index 23.14 (range 20.21 and 28 kg/m^2). Subjects were considered to be in good health by the investigator on the basis of medical history, physical examination including vital sign assessment, 12-lead electrocardiogram, and routine laboratory tests. Concomitant medications (prescription, over-the-counter, or herbal medications), with the exception of up to 3 doses of acetaminophen at 1 g each or less, were prohibited. Smoking and intake of drugs, alcohol (ethanol), coffee or tea were not permitted during the study. Solicitation for compliance with the protocol was based on the Declaration of Helsinki (1964) and its revisions. All subjects gave written informed consent and the local Research and Ethics Committee of Hospital Memorial Guararapes, Jaboatão dos Guararapes, PE, Brazil, approved the clinical protocol. No adverse event occurred.

Drug assay

Plasma digoxin concentrations were measured with the sensitive and selective AxSYM Digoxin II method (LOQ 0.30 ng/mL), which is simple, economic, and straightforward for digoxin quantification in human plasma and is less expensive and laborious than HPLC/MS/MS. As demonstrated in this assay, this method is perfectly suitable for high through-put routines such as PK studies. Immunoassay for digoxin was obtained as a reagent kit (AxSYM Digoxin II) from Abbott Laboratories, USA.

Pharmacokinetics

The mean plasma concentration in ng/mL of digoxin vs time curve obtained after a single oral dose of 0.5 mg digoxin formulation are shown in Figure 1. The mean ± SD digoxin serum concentration was 2.35 ± 0.75 ng/mL, which was 12.26% lower than the level measured (3.1 ± 0.89 ng/mL) by Borges et al. **[19]**. The lower value was obtained by Richards et al., of 1.65±0.6 ng/mL **[26]**. The

peak concentrations ranged between 0.99 and 3.78 ng/mL. The larger value was obtained by White et al., from 1.2 to 4.8 ng/mL **[27]**. The time to reach peak concentration was between 0.5 and 2.0 h, similar results were published by Pähkla *et al*., of between 0.5 and 2.2 h **[28]**. Plasma concentrations achieved at a median t_{max} of 1.0 hour, in accordance with previously published data **[19, 26]**. PK parameters were calculated by OpenOffice calc on the Linux platform. The mean and SD PK parameters of the areas under plasma concentration/time curves (AUCt) in h.ng/mL, and to infinity (AUC∞) in h.ng/mL, half-life (t½) in h, elimination rate constant (ke) in h-1, clearance (CL) and distribution volume (Vd) are presented in Table II. The present study demonstrates large inter-individual differences in digoxin bioavailability. The AUC values of different volunteers differed by more than three-fold, the values ranged from 12.75 to 43.07 h.ng/mL and the coefficient of variation was 32.09%. A similar result was obtained by Pakla et al. **[28]**. The mean of AUC_t (28.44) is lower than that measured by Borges et al. **[19]**, who found 32.91 h.ng/mL. Many authors have published about the large inter-individual differences in digoxin bioavailability **[29-31]**. The mean clearance of digoxin was 3.51 mL/min/kg. This value was larger than the results obtained in previous formal PK studies (2.9 mL/min/kg) and to the population mean clearance of digoxin was 3.09 mL/min/kg. The inter-individual variability in clearance was 23.97% as a coefficient of variation, a value smaller than the result obtained by Yukawa et al. (29.3%) **[30].** The volume distribution (V*d*) mean was 7.58 L/kg (5.47-15.44) total body weight, a similar result was found (7.5 L/kg) **[32]**; also smaller results were obtained in previous formal PK studies (2.01-8.34 L/kg and 6-7 L/kg total body weight**).** The mean plasma half-life time of digoxin was 25.98 ± 12.38 hours, a similar result was found by Moffat et al. (20-25h) **[31]**. Our result is lower than results found by other researchers: 47.5 ± 9 hours **[26]** and 76.79 h **[19]**, and 36 h (range: 30–40 h) **[33]**. The half-life time is prolonged in subjects with renal impairment **[31]**. PK parameters were calculated by two other programs, Pharmkit and Trapule. The values obtained with these programs were compared with the data obtained by BrOffice calc in Linux software Table II. The Trapule program determines only AUC_t, AUC_∞ and $t_{½}$. All parameters were analyzed by paired t-tests, comparing the average of treatment. There was no significant difference between the means of all PK parameters (The two-tailed p value >0.05), with 95% confidence interval, calculated from OpenOffice and Pharmkit. In the comparison with Trapule, a significant difference was found for the AUC_t and AUC_∞. (The two-tailed p value <0.05). There was no significant difference between the means of $t_{½}$ and k_e (The two-tailed p value >0.05).

CONCLUSIONS

In this paper we employed an AxSYM Digoxin II procedure for quantitative analysis of digoxin in plasma. The procedure has high specificity, selectivity, sensitivity and was validated. Digoxin possesses a narrow therapeutic index and shows a large interpatient pharmacokinetic variability, which was verified in this study. The digoxin pharmacokinetic parameters observed in our study are in accordance with those found in the literature in healthy volunteers for a single 0.5mg oral (tablet) dose **[34-36]**. Our results conclude that PK parameters can be calculated by OpenOffice calc, developed in the Linux operating system. Then, the program will help students, teachers and researchers around the world, mainly in the developed countries. It is not necessary to have another computer for calculate PK parameters, because knoppix is a GNU/Linux distribution that boots and runs entirely from CD, DVD or a USB Flash Drive, without prior installation to the hard disk. Knoppix is based on Debian GNU/Linux and includes many useful applications such as OpenOffice, Mozilla along with hundreds of other open-source applications. The next step will be to validate the methodology in OpenOffice Linux in order to publish the program.

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Table I: Accuracy and precision data for digoxin from the pre-study validation in human plasma. Intraday and interday for quality control QCL, QCM and QCH respectively

	Quality Control Low (QCL)	Quality Control Medium (QCM)	Quality Control High (QCH)
Intraday			
Nominal concentration (ng ml^{-1})	**0.9**	**1.9**	**3.2**
mean	**0.92**	**2.11**	**3.3**
SD	**0.085**	**0.043**	**0.025**
Precision (CV%)	**9.21**	**2.05**	**0.75**
Accuracy (%)	**102.31**	**110.99**	**103.18**
interday			
Nominal concentration (ng ml^{-1})	**0.9**	**1.9**	**3.2**
mean	**0.97**	**1.86**	**3.15**
SD	**0.137**	**0.208**	**0.283**
Precision (%)	**14.08**	**11.18**	**8.97**
Accuracy (%)	**108.20**	**97.93**	**98.56**

SD = Standard deviation; CV = coefficient of variation

Table II: Mean (SD) and p value of pharmacokinetic parameters

Parameter	Mean (SD) By OpenOffice	Mean (SD) By Pharmkit	P value	Trapule	P value
Dose (mg)	0.5	0.5		0.5	
AUC_t (h.ng/mL)	30.768 (10.72)	30.768 (10.87)	1.000	30.529 (10.87)	0.0189
AUC_∞ (h.ng/mL)	37.635 (13,145)	37.523 (13.284)	0.5990	35.708 (12.813)	0.0001
$t_{½}$ (h)	25.782 (8.419)	25.773 (8.488)	0.9589	25.7525 (8.402)	0.8532
k_{el} (h-1)	0.030233 (0.01116)	0.030125 (0.01075)	0.6497	0.030132 (0.01075)	0.6777
CL (ml.min.Kg)	3.930 (1.167)	3.947 (1.170)	0.6793	NC	
V_d (l/ Kg)	8.146 (2.165)	8.175 (2.096)	0.7346	NC	
C_{max} (found)	2.721				
t_{max} (found)	0.75				

NC = Not calculated

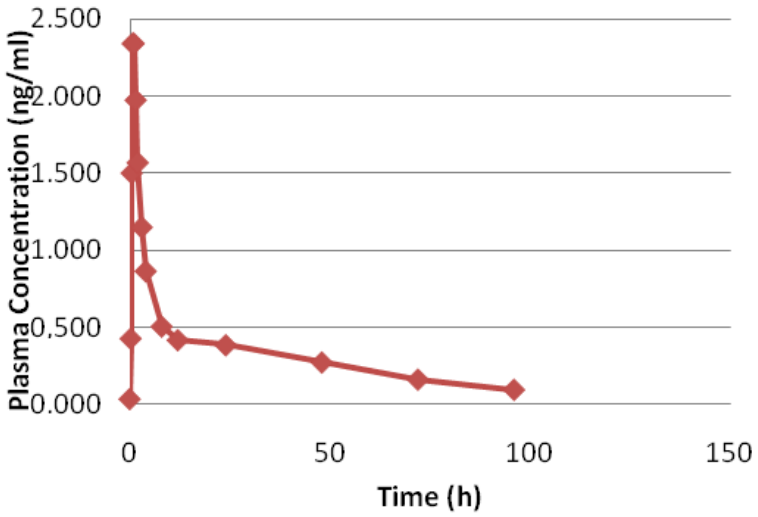

Figure 1: The mean plasma concentration of digoxin vs. time curves obtained after a single oral administration of digoxin