Population pharmacokinetics of benznidazole in adult patients with Chagas disease.

Soy D¹, Aldasoro E⁴, Guerrero L³, Posada E⁴, Serret N⁴, Mejía T⁴, Urbina J.A⁷, Gascón J⁴,⁵

¹Pharmacy Service, Hospital Clinic Barcelona, Spain. ²Institut de Investigació Biomèdica Agustí Pi i Sunyer (IDIBAPS), University of Barcelona, Spain. ³CIBERES (CIBER de Enfermedades Respiratorias, 06/06/0028), Spain. ⁴International Health Service, Hospital Clinic Barcelona, Spain, ⁵ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Barcelona, Spain. ⁶CELLEX Laboratory, University of Barcelona, ⁷Emeritus Investigator, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

Running title: Benznidazole population pharmacokinetics in adult patients.

Keywords: Benznidazole, Chagas disease, Population pharmacokinetics.

Corresponding autor:
Dolors Soy, Pharm D, PhD
Pharmacy Service. Hospital Clínic Barcelona
Villarroel, 170 – Esc 8 sótano
08036 – Barcelona (Spain)
TI: +34.932275479
Fax: +34.932275457
e-mail: dsow@clinic.ub.es
ABSTRACT

Aim: To build a population pharmacokinetic (PopPK) model to characterize benznidazole (BNZ) pharmacokinetics in adults with chronic Chagas disease.

Methods: Prospective, open-label, single-center clinical trial (EudraCT: 2011-002900-34; CINEBENZclinicaltrials.gov number: NCT01755403), approved by the local ethics committee. Patients received 2.5mg/kg/12h (Abarax®, Elea Laboratory, Argentina) for 60 days. Plasma BZN samples were taken at several times along the study and analyzed by HPLC-UV. The PopPK analysis was done with NONMEMv.7.3. Demographic and biological data were tested as covariates. Intraindividual, interoccasion and residual variability were modeled. Internal and external validations were completed to assess the robustness of the model. Later on, simulations were performed to generate the BNZ concentration-time course profile for different dosage regimens.

Results: A total of 358 plasma BZN concentrations from 39 patients were included in the analysis. A one-compartment-PK-model characterized by clearance (CL/F) and apparent volume of distribution (V/F) with first order absorption (Ka) and elimination, adequately described the data (CL/F: 1.73 L/h; V/F: 89.6 L; Ka: 1.15 h⁻¹). No covariates were found to be significant for CL/F and V/F. Internal and external validation of the final model showed adequate results. Data from simulations revealed that a dose of 2.5mg/kg/12h might lead to overexposure in the most of the patients. A lower dose (2.5mg/kg/24h) was able to achieve trough BNZ plasma concentrations within the accepted therapeutic range of 3-6 mg/L.

Conclusion: A population PK model for BNZ in adults with chronic Chagas disease has been developed. Dosing simulations showed that a BNZ dose of
2.5 mg/kg/24h would adequately keep BNZ trough plasma concentrations within the recommended target range concentrations for the majority of patients.
Chagas disease is a zoonotic disease endemic in Latin America, particularly in poor rural areas of Mexico, Central America and South America (1). In recent last years, the disease and its transmission are not longer limited to endemic areas and it has turned into an emerging global public health problem mainly due to migratory flows (2). Its etiological agent is *Trypanosoma cruzi*, a hemoflagellate protozoan (family Trypanosomatidae, order Kinetoplastida), whose life cycle involves obligatory passage through vertebrate hosts (mammals, including humans) and invertebrate hosts (hematophagous triatomine bugs) in a series of stages (3).

The disease has two phases: an initial acute phase, which is usually asymptomatic and a lifelong chronic phase, which in 60 to 70% of patients is clinically silent, but 30 to 40% of them will develop in years or decades heart problems (20 to 30%), digestive problems, or a combination of both (10 to 15%). Acute Chagas disease treatment is highly effective whereas the effectiveness of the antiparasitic treatment to chronically infected patients has been a subject to controversy for years. Nevertheless, lately the evidence supporting trypanocidal treatment during chronic stage of the disease has increased considerably (4–6).

So far two compounds are available for etiological treatment of Chagas disease: benznidazole and nifurtimox, though benznidazole (BNZ) is the most commonly used globally. To date, limited data on benznidazole pharmacokinetics are available (7–9). These studies showed that BNZ has good oral bioavailability, an apparent volume of distribution of 0.56 L/kg, and an
elimination half-life of 12 to 15 h (7, 8). Based on these investigations, the
current recommended dosage of BNZ for the treatment of T. cruzi infection in
humans is 5 mg/kg/d, divided in two doses (2.5 mg/kg/12h) for 60 days. The
trypanocidal concentration of BNZ in-vitro studies ranges from 3 to 6 mg/L (8),
and concentrations close to 20 mg/L had been described to be possibly related
to a higher risk of toxicity, although in a recent study no relationship was found
between the BNZ serum concentration and adverse drug reactions (10).

Giving the limited available data on benznidazole human pharmacokinetics
(7, 8), population pharmacokinetic (popPK) modeling may play an important role
in assessing the effect of demographic and physiological factors on drug
exposure. With the knowledge of the population mean and inter-individual
variability of the PK parameters, it is feasible to: (a) simulate credible responses
to different drug dosing schemes and (b) customize dosage regimens to satisfy
an specific criterion (11, 12).

The main goal of this study was to characterize the pharmacokinetics of
benznidazole in adult patients with Chagas disease, using a model-based
approach, and identify those factors that contribute to its PK variability to
establish new drug dosing schemes.

Materials and methods

Patients

The CINEBENZ study is a prospective, open-label, single-center clinical trial
conducted in the Tropical Medicine Unit in the Clinic Hospital of Barcelona and
sponsored by CRESIB (Barcelona Centre for International Health Research).
The protocol was approved by the ethics committee of the Clinic Hospital of
Barcelona and AEMPS (the Spanish Agency of Medicines and MedicalDevices), was registered in the European Clinical Trials Database (EudraCT:2011-002900-34) and clinicaltrials.gov website (CINEBENZ clinicaltrials.gov number: NCT01755403), and was conducted in accordance with theDeclaration of Helsinki and national and institutional standards. Before inclusionin the study, all patients provided written informed consent.

Subjects eligible to participate in the study need to meet the inclusion criteria
to be at least 18 years old, diagnosed with Chagas disease by two different T.cruzi serologic tests and not to have received treatment before. Exclusioncriteria were hypersensitivity to BZN, close follow-up impossibility,immunodeficiency (HIV, cancer, prolonged corticoid treatments, any otherprimary or secondary immunodeficiency), hepatic or renal impairment,pregnancy or lactation. Enrolled subject received treatment with BNZ 2.5mg/kg/12h for 8 weeks, with maximum 400mg/day (Abarax®, Elea Laboratory,Argentina).

Data

Demographic characteristics such as age, gender, total body weight, height,index body mass (total body weight/heigth\(^2\)) were recorded in the firstevaluation. Clinical laboratory measurements, such as serum creatinine andcreatinine clearance (CL\(_{cr}\)), estimated according to the Cockcroft-Gault formula(13), hematocrit, total serum proteins and total bilirubin were documented inevery visit during treatment. Drug adherence was recorded every follow-up visitduring treatment by drug pill counting by the investigator.
Blood BZN samples were taken at different times. They were collected as follows: (i) 1h; (ii) between 3 and 6h; (iii) and between 6 and 12h after the oral morning dose (observed by an investigator) on treatment days +1 (first dose) and +15 (at steady-state conditions). After the last treatment dose, several samples were also drawn: (i) one point between 3-12h; (ii) another between 12 and 24h; and (iii) an extra sample between 24 and 36h. Additional trough samples were collected at day +30 and +45, or at unscheduled visits during routine clinical follow-up, mainly for controlling drug adherence. Time and dose deviations were recorded and taken into account in the PK analysis.

All blood samples were centrifuged at 5000 g for 10 min. Plasma was separated and stored at −40°C until analysis by high performance liquid chromatography with ultraviolet-visible detection (HPLC-UV) according to a previously published methodology developed in our research laboratory (14). Linearity was found in the range of 1.6-100 mg/L. The analytical error between replicates was less than 6%. The quantitation and detection limits of the assay were 1.6 and 0.8 mg/L, respectively.

Pharmacokinetic modeling

Non-linear effects modeling was performed using NONMEM v.7.3 (Icon Development Solutions, USA)(15), following a three step strategy: (a) basic population model selection, (b) covariate selection, and (c) model validation. The first-order conditional estimation with interaction (FOCE-I) method was used for parameter estimation.

Models of one and two open compartments with first-order absorption and first-order elimination were evaluated. Interindividual (IIV) and interoccasion
(IOV) (16) variability were assumed to be log-normally distributed. Regarding IOV each sampling day was defined as one occasion.

Consider for a generic PK parameter the following model:

\[ P = \theta_P \ast \exp(\eta_P + \kappa_P) \]

where \( \theta_P \) is the population mean of \( P \) (\( P = CL, V \)) and \( \eta_P \) and \( \kappa_P \) are the random effects capturing the IIV and IOV variability of \( P \), respectively. Additive, proportional and combined error models were tested for residual variability (RV) (which comprises measurement and model error) on drug concentrations.

Goodness of fit for a given model was assessed by: (a) changes in the NONMEM minimum objective function value (OFV): -2log-likelihood; (b) plots of population and individual Bayesian predicted concentrations vs. observed BNZ concentrations; and weighted residuals and conditional weighted residuals vs. observed concentrations and time (17, 18); and (c) changes in the standard error of parameter estimates (precision). The difference in -2log-likelihood between two hierarchical models (log-likelihood ratio test) is asymptotically \( \chi^2 \)-square distributed with degrees of freedom equal to the difference in number of model parameters. A significance level of 0.05% and 0.01% denoted a significant improvement of fit (drop in the OFV by more than 3.841 and 6.64, respectively) for a one-parameter difference. R v3.1.1 (the R Foundation for Statistical Computing, Vienna, Austria) and the package Xpose v4.5.0 (19), was used to guide the model building process. Pearl-speaks-NONMEM (PsN) v.3.7.3 was used for automation throughout the modeling process (20).

In a second step, all reasonable demographic and biological factors were tested for inclusion as covariates in the basic population PK model to explain IIV
variability. They were tested in NONMEM by using “scm” approach. Continuous covariates were generally assessed as follows:

\[ P_j = P_{\text{POP}} \times \left( \frac{\text{Cov}_j}{\text{Cov}_{\text{MEAN}}} \right)^{\theta_{\text{COV}}} \]

where \( P_j \) is the PK parameter for the \( j^{\text{th}} \) patient and a given covariate value, \( P_{\text{POP}} \) is the typical value of a PK parameter corresponding to the mean value of the covariate of interest in the population, \( \text{Cov}_j \) is the value of that covariate for the \( j^{\text{th}} \) patient, \( \text{Cov}_{\text{MEAN}} \) is the mean of the covariate in the population and \( \theta_{\text{COV}} \) represents the scaling factor for the influence of that covariate. Categorical variables were included in the model as expressed below:

\[ P_j = P_{\text{POP}} + \theta_{\text{COV}} (1 - \text{Cov}_i) \]

where \( P_j \) is the PK parameter for the \( j^{\text{th}} \) patient, \( \text{Cov}_i \) is a numeric index value (in that case: 1 for the reference category or 0 for the comparative category), \( P_{\text{POP}} \) is the typical value of a PK parameter for the reference covariate values (ie: \( \text{Cov}_i \) equals 1) and \( \theta_{\text{COV}} \) is the multiplicative factor for the influence of this covariate on the PK parameter.

Covariates were first entered one by one into the population model and then by the cumulative forward inclusion/backward elimination procedures. Each covariate investigated in NONMEM was retained if it led to a significant improved fit. Improvements to the model were evaluated by: (a) biological plausibility of the covariate, (b) graphical displays based on the agreement between the observed (OBS) and predicted drug concentrations, (c) the uniformity of the distribution of the residuals, and (d) the log-likelihood ratio test between two-nested models. Covariates were kept in the model if they yielded a significant level of 5% (reduction in the log-likelihood ratio test of 3.841 points). A significant level of 1% (increase in the log-likelihood ratio test of 6.635 points)
was employed during the backward elimination step. A decrease of at least 10% in IIV associated with a specific pharmacokinetic parameter was considered clinically relevant for the inclusion of that specific covariate. The extent of Bayesian shrinkage in the PK parameters was evaluated for each parameter in the final model (21).

**Model evaluation**

The internal validation of the PK model was assessed by graphical and statistical methods, including visual predictive checks (vpc) (22). Bootstrap resampling technique (with replacement) was used to build confidence intervals (CIs) of pharmacokinetic parameters to assess their stability and evaluate the robustness of the final model (23). It was performed in PsN v.3.7.3 (University of Uppsala, Sweden) (20). The final model was fitted to the replicate data sets (200 data sets), and parameter estimates for each of them were obtained. The mean values (and the lower and upper limits of the 95% CI) of the parameters obtained were compared with those estimated from the original data.

The external predictive performance of the PK model was assessed by analyzing data from new individuals (validation data set) treated with BNZ in similar conditions to the study population (index data set) (24). Individual predicted BNZ concentrations for all sampling times were obtained by Bayesian estimation ("posthoc" subroutine of NONMEM without the estimation step) setting population PK parameter values (mean PK parameters, interindividual and residual variability) to population values previously obtained in the index data set. The performance of the Bayesian analysis was evaluated by comparison of the observed (OBS) concentrations with the population predicted
(PRED) and individual predicted (IPRED) concentration values. Bias and precision were calculated and expressed in terms of percentage prediction error (IPE% and PPE% for IPRED and PRED, respectively) and absolute percentage prediction error (IAPE% and PAPE% for IPRED and PRED, respectively), (25).

Dosage regimen simulations:

Monte Carlo simulations (1000 simulations) were performed using the final model for BNZ to investigate the presumed steady-state drug concentrations for new dosing regimens. Following the presumption that the effectiveness of BZN depends on sustaining a concentration within the trypanocidal range (3-6 mg/L) (8), the recommended dose regimen of 2.5 mg/kg/12h and a once daily dose of 2.5 mg/kg and 5 mg/kg were evaluated.

However, considering the recent finding of Bustamante et al. (26) that *T. cruzi* infection can be cured (in mice) using 13 doses of BNZ given at 5 days intervals, and the knowledge of the *T. cruzi* life cycle duration (1, 26) a dose regimen of 5 mg/kg of BZN 5 days apart was also tested by simulating 1000 patients from our final population PK model.

Influence of body weight on dosage:

Based on the theory-based PK modeling approach, some relationships may be included in the popPK model even without being statistically significant. In this context, the inclusion of allometric scaling on the PK parameters could help to have an idea of the connection between body size and drug PK; particularly, the influence of body weight on optimal dosing. BNZ is dosed on kilograms basis and thus, this may have a certain impact on drug exposure. With the
intention to understand the influence of total body weight on BNZ PK, we decided to include this effect in the model to assess the fraction of patients’ over- and underexposed at extreme weights; 43 Kg and 100 Kg. These values correspond to the minimum and maximum total body weight in our cohort of patients.

Hence, 1000 additional simulations were done using a new popPK model (WT-popPK model) including the allometric scaling on the PK parameters, as follows:

\[
\frac{\text{CL}_{i}/F}{\text{TVCL}} = (\frac{\text{WT}_{i}}{\text{WT}_{\text{mean}}})^{0.75}; \quad \frac{\text{V}_{i}/F}{\text{TVV}} = (\frac{\text{WT}_{i}}{\text{WT}_{\text{mean}}})^{1}
\]

where: \(\text{CL}_{i}/F\) and \(\text{V}_{i}/F\) are the individual total BNZ clearance and apparent volume of distribution for the \(i^{\text{th}}\) individual, TVCL and TVV are the “typical” population values of the drug clearance and apparent volume of distribution, \(\text{WT}_{i}\) is the weight of the \(i^{\text{th}}\) individual and \(\text{WT}_{\text{mean}}\) is the average weight of all patients included in the study. The allometric exponents were fixed to the values obtained from the literature (27, 28).

Statistical analysis:

Statistical analysis was performed using S-Plus 6.1 (S-Plus 6.1 for Windows Insightful Corporation, Seattle WA. Insightful Corporation, 2002). Mean, median, standard deviations (SD), 90% confidence intervals [90% CI] and quartiles (q) were calculated for continuous variables. Student t-test was used for comparing normally distributed variables, U-Mann Whitney test was used for comparing non-normally distributed variables.
Results are expressed as absolute and relative frequencies for categorical variables and Chi-square test was used for comparing them. The significance level for all the analyses was defined as p<0.05.

RESULTS

Subjects and samples:

Fifty-two subjects met the enrolment criteria and signed the informed consent. One subject withdrew the consent before treatment was started. Two subjects were excluded from the PK analysis due to early treatment discontinuation because of adverse reactions. Eventually 49 subject’s samples were analysed. The majority of patients were from Bolivia (96%), women (71%) and the median age was 36 (range from 19 to 55).

Data from 39 subjects were use in the model construction (Table 1: index data set) and 10 for validation (Table 1: validation data set). A total of 358 plasma BZN concentrations ranging from 0.56 to 28.94 mg/L, were used during the analysis (index data set). On average, 9.1 samples were collected per patient during the whole study. Patients’ age and body weight (index data set) ranged from 19 to 55 (mean: 37.15 years) and 43 to 105 (mean: 70.55 kg), respectively. Mean values (± SD) in this group were: CLcr: 124.4 ± 25.1 mL/min; hematocrit: 0.40 ± 0.04%; total serum proteins: 72.9 ± 4.2 mg/dL. The median global adherence (index data set + validation data set) was 99.2% with only two cases with adherence lower than 85%.
Population pharmacokinetic model:

The pharmacokinetics of BNZ was best described by a one-compartment open linear model with first order absorption and first order elimination, typified by apparent clearance (CL/F), apparent volume of distribution (V/F) and population absorption rate constant (Ka). A second distribution compartment did not improve the model fit. BNZ was administered as an oral tablet and the bioavailability of the drug could not be estimated. Hence, the model estimates are the values of apparent clearance (CL/F) and volume of distribution (V/F), where F represents oral bioavailability. The available data did not support the estimation of Ka and thus it was set to a fixed value obtained from the literature (8). Interindividual variability (IIV) was incorporated in CL/F and V/F. Interoccasion variability (IOV) in CL/F significantly reduced the OFV ratio test (Δ 97 points; P > 0.001). Residual variability (RV) was characterized by a combined error model with a proportional part of 19.1% (expressed as coefficient of variation [CV%]) and an additive part of 0.57 mg/L (expressed as standard deviation [SD]).

During covariate model selection, the effect of age, gender, total body weight, index body mass, CL\textsubscript{cr}, total serum proteins and total bilirubin were tested on relevant pharmacokinetic parameters (CL/F and V/F). The results showed that none of the covariates (age, gender, total body weight, index body mass, total bilirubin, total serum proteins, hematocrit and CL\textsubscript{cr}) significantly influenced BNZ CL/F (reduction of the NONMEM objective function value (p<0.05)). Inclusion of covariates in V/F failed to cause a significant drop in the OFV ratio test as well.
An overview of goodness-of-fit-plots for the final PK model is given in Fig. 1. A good accordance between observed and population/individual predicted concentrations is observed. Residual error plots showed no systematic deviation over time (Fig 1). The mean of the CWRES was 0.085, close to zero, indicating the ability of the estimation method to fit the model to the data. The model parameters had reasonably levels of η-shrinkage for CL/F (13.1%) and V/F (34.7%). The magnitude of the ε-shrinkage was 27.7%.

An overview of the pharmacokinetic parameter estimates from the final population PK model is presented in Table 2.

**Model validation:**

As shown in Fig. 2, results from the visual predictive check showed that practically all observations dropped into the 95% confidence intervals (CI). The statistical distributions of the parameter estimates obtained from the bootstrap analysis are shown in Table 2. Median values of the parameter estimated from the bootstrap are in good agreement with the NONMEM point estimates, and the 95% CIs were reasonably narrow, demonstrating satisfactory precision. Visual and numerical predictive checks demonstrated good predictive performance of the final pharmacokinetic model.

Regarding the predictive performance of the model, the validation data set included 10 new patients whose demographic and clinical characteristics are displayed in Table 1. A total of 96 plasma BZN concentrations ranging from 0.57 – 13.21 mg/L, were used for external validation of the full model. On average, 9.6 samples were collected per patient. Patients’ age and body weight ranged from 26 to 52 (mean: 38.8 years) and 51 to 91 (mean: 68.4 kg),
respectively. Mean values (± SD) in this group were: CL_{cr}: 125.7 ± 23.1 mL/min; hematocrit: 0.38 ± 0.03%; total serum proteins: 70.5 ± 1.8 mg/dL. Median bias and precision for the MAP Bayesian estimates (IPRED) resulted in 0.76% and 9.04%, respectively, much better than those values obtained from the population PK-model based estimates (PRED), which were −32.73% and 36.19%, respectively (Fig. 3).

Dosage regimen simulation at steady state conditions:

Results from simulations are displayed in Table 3. The usual dose regimen of 2.5 mg/kg/12h, for an average patient of 70 kg of weight, would be enough to attain the target trypanocidal concentration during the whole inter-dose interval (12 hours) in almost all treated subjects (95% of the patients; quartile 5^{th}: q_5: 3.02 mg/L at 10h), since the simulated trough median [90% confidence interval: CI] at steady-state resulted to be 7.53 [2.81 – 17.48] mg/L.

Simulations considering other dose regimens such as (a) 2.5 mg/kg/day q.d., (b) 5 mg/kg/day q.d. and (c) an extended dosing interval of 5 mg/kg of BZN 5 days apart were also done. The median [90% CI] trough BNZ plasma concentrations at the last day of treatment (steady-state conditions) from 1000 simulated profiles are shown in Table 3. For an average patient of 70 kg, the results indicated that once daily dose regimens: 2.5 mg/kg/day q.d. and 5 mg/kg/day q.d. were able to attain the target trypanocidal concentration during the whole inter-dose interval of 24 hours. In contrast, for the extended dosing interval of 5 mg/kg of BZN 5 days apart the drug levels fell below the minimum trypanocidal level after 48 hours (median [90% CI]: 2.83 [0.54-6.36] mg/L). With
this extended regimen, the median [90% CI] peak BNZ concentrations 2h after
dose were 7.49 [2.81-20.36] mg/L.

Influence of body weight on dosage:
The final popPK model including the allometric relationship between body
weight and PK parameters (WT-popPK) did not improve the fit. The population
PK estimates were very similar to the original final popPK model (without
considering weight as an influential covariate). In the WT-popPK model the final
estimates of fixed effect parameters were: CL/F = 1.75 L/h, V/F = 95.3 L,
respectively. Ka was fixed to 1.15. Lognormal IIV in CL/F and V/F resulted in
values of 34.1% and 77.3%, respectively. Interoccasion variability was only
retained for CL/F (30%), as in the original model.

Results of simulations after considering the influence of body weight on BNZ
dosing and drug exposure are presented in Table 3.

DISCUSSION
The most widely available drug for treating Chagas disease is benznidazole,
but little information exists on its pharmacokinetics. Recently a study on
population PK of benznidazole in children was published (29) but to our
knowledge, the present population PK study is the first to examine benznidazole
plasma concentrations in adult subjects with Chagas’ disease.

In this study we used NONMEM v.7.3 (15) to characterize the PK of the drug
and to investigate quantitative relationships between the pharmacokinetic
parameters and physiological and/or demographic features, in subjects with
Chagas disease treated with benznidazole. The data collected in this study was
best described by an open one-compartment PK model with first-order absorption and elimination, characterized by CL/F, V/F and Ka, which is in accordance with previous data (7, 8). The population estimates for CL/F and V/F were 1.73 L/h and 89.6 L, respectively; again in line with previously published results. Interindividual variability (IIV) was incorporated in CL/F and V/F and interoccasion variability in CL/F significantly reduced the OFV (P = 0.01). Our work showed a high interindividual variability for PK parameters, 42% in CL/F and 39.3% in V/F [CV%]. However, neither CL/F nor V/F was significantly influenced by the studied demographic or physiopathological factors. Goodness-of-fit plots and simulation-based diagnostics showed that the model described the data adequately.

Previous BZN PK studies were conducted in healthy volunteers (7) or patients with chronic Chagas disease (8) and have been analyzed (posthoc analysis) on a population approach basis by Altchech et al. (29). In these studies, combined data from healthy (single dose) and patients (multiple doses) led to a value of CL/F of 0.0301 L/h/kg (2.17 L/h for a patient of 70 kg) and a typical half-life value of 12.77 h. In the present study, performed at steady-state conditions in 39 adults with chronic Chagas disease, the estimated terminal drug half-life was about 36 hours, which is quite longer than the previous estimates. We presume this might be related to the different characteristics of the study populations, mainly patient condition vs. healthy and adults vs. pediatrics.

Regarding the inclusion of IOV on CL/F after repeated oral BNZ, and taking into account the excellent adherence to treatment seen in our cohort of patients all along the study, with a mean (± SD) drug adherence of 98.12 ± 7.53%, it is
possible to hypothesize that variability might be attributed to differences either in CL or in bioavailability (F). Unfortunately, intravenous BNZ is not available in the market and hence it is not possible to use it as a reference form to estimate the “true clearance: CL”. Thus, it was not feasible to discriminate in our model if IOV was related to CL or F since after oral drug administration CL and systemic availability are confounded (16).

A major goal of population PK studies is to identify those covariates which explain the variation in the between-patient variance component (30). However, none of the covariates tested in this study showed a significant impact on oral BNZ pharmacokinetics in our sample. Besides, another valuable feature of population PK studies is the opportunity to perform Monte Carlo simulations (11). This is a very powerful method used to forecast therapeutic outcomes and design adequate dosing regimens (effective and safe) (12). Therefore, we applied this methodology to theoretically determine the suitability of several potential BNZ dosage regimens which could be further tested in clinical trials (31). However, before carrying out any simulation to assist us in selecting an optimal initial dosage regimen, it should be previously established that the described population PK model is predictive (24). External validation was conducted by assessing the ability of the population model to predict concentrations in a separate group of 10 patients with chronic Chagas disease. The model was evaluated by means of bias and precision showing mean values within acceptable limits (0.76% and 9.04%, respectively) and supporting the validity and the further utility of this population PK model.

To date, no other pharmacokinetics/pharmacodynamics relationship or target exposure values other than keeping BNZ trough concentrations within or
above the *in vitro* trypanosomicidal range (3-6 mg/L) (8) have been established for optimizing treatment of Chagas disease. However, it must be noted in that in several studies in the last 25 years (32–35) have shown that in many cases there is no correlation between the *in vitro* susceptibility of a given *T. cruzi* strain to benznidazole or nifurtimox and the efficacy of antiparasitic treatment with these drugs in experimental animals or human patients. This lack of correlation is explained by the multiple physicochemical and biochemical barriers to access of any drug to their potential cellular targets in an intact organism, a fact that may contribute to the known variability of the outcome of etiological treatment (36).

Data from the original study (popPK) showed that the standard 5 mg/kg/day of BZN divided in two doses (2.5 mg/kg/12h) lead to a median [90%CI] trough BNZ concentration of 7.53 [2.81 - 17.48] mg/L, for an average patient of 70 kg. As expected, for 2.5 mg/kg/12h, the simulated trough concentration of BNZ, were below 3 mg/L at 12h in only 5% of patients, consistent with the observed data in this study. Looking deeper into our dataset we noted that only 5.4% of the observed (real) BNZ trough concentrations were below 3 mg/L, 20% of them were within the optimal range (3-6 mg/L) but the most of them, 74.54% above 6 mg/L. These results support the views of other authors (26, 29), which suggested that standard treatment protocols using BNZ might be significantly overdosing patients and therefore studies with lower doses in adults might be justified.

Therefore, by means of Monte Carlo simulations, a new 50% reduced total dose regimen of 2.5 mg/kg once daily was tested. Results from this test showed that this new proposed dose could keep median BNZ concentrations above 3
mg/L for the whole interval of 24h. It should be noted that for this dose regimen
sub-therapeutic trough concentrations (< 3 mg/L) were reached at 18 hours of
BNZ administration in only 15% of patients. As regards to the once daily dose
regimen of 5 mg/kg/24h, median trough concentrations were always above the
upper limit of the therapeutic range (> 6 mg/L) and only in 4-5% of cases, BNZ
concentrations would feel below 3 mg/L during the last 4 hours of a dose
regimen of 24h. With this dosing regimen the most distressing point would be
the BNZ concentrations achieved at earlier times after drug administration
(median \(C_{\text{max}}\) [90% CI: 7.49 [2.87-20.36] mg/L just 2-3 hours after dose, since in
5% of patients it could reach values close to the toxic level of 20 mg/L. Even
though our previous work (37) showed that BNZ concentrations might not be
related to the appearance of serious drug effects, we believe such high drug
concentrations are neither desirable nor needed in patients with Chagas
disease. Finally, simulations for 5 mg/kg/5 days showed median trough
concentrations far below the recommended target of 3 mg/L (Table 3). As
stated before, we chose this dose schedule by imitating the dosage regimen
used by Bustamante et al. (26) in their study in mice. However, the
effectiveness of these regimens in which minimum BNZ concentrations remain
below the suggested goal are still controversial. They are based on the fact that
drug efficacy would be related to the maximum BNZ concentration \(C_{\text{max}}\). Up to
now, this assumption has not been proved in humans and making it difficult to
recommend these extended intervals (> 24 h) in Chagas’ treatment.

In our final popPK model, total body weight (WT) did not find a significant
influence on any PK parameter. In order to investigate a possible weakness due
to this fact, we conducted additional simulations taking into account the
theoretical relationship between WT and PK, based on the allometric scaling
described in many references in the literature (27,28). Results from simulations
show that the usual dose regimen of 2.5 mg/kg/12h would allow achieving the
target of 3 mg/L during the whole inter-dose interval in almost all of the treated
subjects. Taking into account the influence of total body weight on the
administered dose, the simulated trough BNZ median [90% CI] concentration at
steady-state resulted to be 7.73 [2.88-17.61] mg/L and 10.05 [4.06-23.33] mg/L
for an average patient of 43 kg and 100 kg of total body weight, respectively
(Table 3). However, probably more important is the fraction of patients over-
and underexposed to BNZ, based on the weight-based dosing, since these are
the patient on risk. These data are displayed in Table 3. Simulations with 2.5
mg/kg/12h and 5 mg/kg/24h of BNZ showed that the percentage of patients with
trough BNZ concentrations higher than 6 mg/L was between 70-80%. No
significant differences within groups of different total body weight were seen.
These high concentrations are probably excessive and we might consider they
could be toxic (in 5% of patients; upper limit of the CI higher or equal to 20
mg/L). From this point of view, a reduction in the total BNZ daily dose might be
considered. The fraction of overexposed patients decreased to 25-34% for a
dosage regimen of 2.5 mg/kg/24h with no risk of achieving toxic concentrations.
Nonetheless, with this dose regimen, the risk of BNZ under-exposition (median
trough concentrations below 3 mg/L at 24h) increased up to around 30% of
patients. Interestingly, and regardless of the total body weight, only 15% of
patients would show sub-therapeutic trough BNZ concentrations at 18 hours of
BNZ administration. Total body weight had no impact on the fraction of patients
under-exposed since no significant differences were seen between groups (p>0.05) (Fig.4).

A potential limitation of any population PK study could be the lack of treatment adherence in some patients. Nonetheless, this limitation does not affect our results because the median global adherence in our cohort of patients was 99.2%, with only two cases with values lower than 85%. Another drawback to consider is the impossibility to determine the absolute BNZ bioavailability and the influence of the IOV on it since there is no intravenous formulation to use as a comparator to the oral one.

The current study represents a first step toward developing optimal oral BNZ dosing schedules for the treatment of Chagas disease patients on the basis of a better knowledge of BNZ pharmacokinetics in adults.

In conclusion, this is the first population PK study in adults demonstrating that the standard doses of oral BNZ (2.5 mg/kg/12h) may be significantly overdosing patients. In addition, the results from this study might assist the optimization of BNZ dosing regimens and highlight the rational of proposing a lower BNZ dose (2.5 mg/kg/24h). Additional clinical trials in adults with this lower BNZ dose, looking for safety and mainly efficacy, might be warranted before recommending its use in Chagas disease treatment.
Acknowledgements

This work was supported by Fundación Mundo Sano. ISGLOBAL Research group receives funds from the Agència de Gestió d’Ajuts Universitaris i de Recerca (AGAUR) grant number 2014SGR26, and from the Tropical Disease Cooperative Research Network (RICET), grant number RD12/0018/0010.

Conflict of interest

The authors have no conflicts of interest to declare.
REFERENCES


Table 1: Blood samples and patients’ characteristics for both groups of patients: the index data set and the validation data set. CL<sub>cr</sub>: creatinine clearance (mL/min).

<table>
<thead>
<tr>
<th></th>
<th>Index data set</th>
<th>Validation data set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma samples</td>
<td>358</td>
<td>96</td>
</tr>
<tr>
<td>Plasma concentrations range (mg/L)</td>
<td>0.56 – 28.9</td>
<td>0.57 – 13.21</td>
</tr>
<tr>
<td>No. (females/males)</td>
<td>39 (26/13)</td>
<td>10 (9/1)</td>
</tr>
<tr>
<td>Plasma samples/subject</td>
<td>9.1</td>
<td>9.6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.1 ± 7.5</td>
<td>38.8 ± 8.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.5 ± 14.5</td>
<td>68.4 ± 11.3</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>27.8 ± 4.4</td>
<td>26.9 ± 4.4</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>0.40 ± 0.04</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.70 ± 0.14</td>
<td>0.67 ± 0.10</td>
</tr>
<tr>
<td>CL&lt;sub&gt;cr&lt;/sub&gt; (mL/min)</td>
<td>124.4 ± 25.1</td>
<td>125.7 ± 23.1</td>
</tr>
<tr>
<td>Total serum proteins (mg/dL)</td>
<td>72.9 ± 4.2</td>
<td>70.5 ± 1.8</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.3</td>
</tr>
</tbody>
</table>
Table 2: Benznidazole population pharmacokinetic parameter (PPK) estimates for the base and final models and bootstrap results for the final model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Base/final PPK model estimate</th>
<th>Mean [(95% CI) bootstrap results(^{(a)})]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pharmacokinetic parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>1.73</td>
<td>1.73 [1.54 – 1.92]</td>
</tr>
<tr>
<td>V/F (L)</td>
<td>89.6</td>
<td>92.54 [45.56 – 133.64]</td>
</tr>
<tr>
<td>Ka (h(^{-1}))</td>
<td>1.15 FIX</td>
<td>----</td>
</tr>
<tr>
<td><strong>Interindividual (IIV) variability in(^{(b)}):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F</td>
<td>33.4</td>
<td>33.1 [17.3 – 44.0]</td>
</tr>
<tr>
<td>V/F</td>
<td>68.8</td>
<td>72.3 [25 – 94.2]</td>
</tr>
<tr>
<td><strong>Interoccasion (IOV)variability in(^{(b)}):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL/F</td>
<td>29.5</td>
<td>29.9 [20.4 – 36.4]</td>
</tr>
<tr>
<td><strong>Residual variability (RV):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\sigma_1^2) (mg/L)</td>
<td>0.57 ± 0.19</td>
<td>0.56 [0.12 – 1.27]</td>
</tr>
<tr>
<td>(\sigma_2^2) (CV%)</td>
<td>19.53</td>
<td>17.1 [24.1 – 57.1]</td>
</tr>
</tbody>
</table>

\(^{(a)}\) Derived from 200 successful bootstrap sample runs. \(^{(b)}\) Estimates of interindividual/interoccasion variability are expressed as coefficient of variation of variance values (CV\(\omega^2\)).
Table 3: Trough BNZ plasma concentrations in mg/L (expressed as median [90% CI]) at steady-state conditions, and percentage of patients with trough BNZ concentration > 6 mg/L or < 3 mg/L, resulting after simulating 1000 PK profiles for different dosing schemes and total body weight.

<table>
<thead>
<tr>
<th>Dosing Scheme</th>
<th>Median [90% CI]</th>
<th>43 kg</th>
<th>70 kg</th>
<th>100 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2.5 mg/kg/12h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[BNZ] &gt;6 mg/L at 12h (%)</td>
<td>69.5</td>
<td>67.7</td>
<td>81.8</td>
<td></td>
</tr>
<tr>
<td>[BNZ] &lt;3 mg/L at 12h (%)</td>
<td>5.3</td>
<td>5.5</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td><strong>2.5 mg/kg/24h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[BNZ] &gt;6 mg/L at 24h (%)</td>
<td>22.5</td>
<td>27.5</td>
<td>34.2</td>
<td></td>
</tr>
<tr>
<td>[BNZ] &lt;3 mg/L at 24h (%)</td>
<td>33.6</td>
<td>25.5</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td><strong>5 mg/kg/24h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[BNZ] &gt;6 mg/L at 24h (%)</td>
<td>70.2</td>
<td>80</td>
<td>79.2</td>
<td></td>
</tr>
<tr>
<td>[BNZ] &lt;3 mg/L at 24h (%)</td>
<td>7.8</td>
<td>4.5</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td><strong>5 mg/kg/5days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[BNZ] &gt;6 mg/L at 168h (%)</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>[BNZ] &lt;3 mg/L at 168h (%)</td>
<td>97.7</td>
<td>96.6</td>
<td>94.2</td>
<td></td>
</tr>
</tbody>
</table>

[BNZ]: BNZ concentration
**Legend for figures**

Fig. 1: Goodness of fit plots for the final population PK model. Left upper panel: plot of population predictions (PRED) vs. observed BNZ concentrations (OBS); Right upper panel: plot of individual population predictions (IPRED) vs. OBS; left lower panel: individual weighted population residuals (IWRES) vs. IPRED; right lower panel: conditional weighted residuals (CWRES) vs. time. Black dashed line: line of identity (upper panels) or target line (lower panels); red thick line: line indicating the general data trend. Concentrations (OBS, PRED and IPRED) are in mg/L; time is in hours.

Fig. 2: Visual predictive check (VPC). Comparison between the 5th, 95th (dashed lines) and 50th (full line) percentiles obtained from 1000 simulations and the BNZ observed plasma concentrations (open circles) for adults Chagas patients.

Fig. 3: Box-plots of percentage error (PE) and absolute percentage error (APE) of the validation data set as a measure of bias and precision, respectively. Ordinate: in percentage. The white band in each error box marks the 50th percentile (dashed line); the box boundaries are at the 25th and 75th percentiles, and the limits of the whiskers are at the 10th and 90th percentiles. Other horizontal lines are “outliers”, i.e. values outside the 10-90-percentile range.

IPE: Percentage error for the individual predictions; PPE: Percentage error for the population predictions. IAPE: Absolute percentage error for the individual predictions; PAPE: Absolute percentage error for the population predictions.
Fig. 4: Median BNZ concentrations vs. time at steady state (from 1000 simulated profiles) for a dose regimen of 2.5 mg/kg/24h and different total body weight: 43 Kg (dashed line), 70 Kg (solid line) and 100 Kg (dotted line). Y-axis: BNZ conc (mg/L); x-axis: time (h). Trypanosomicidal range concentrations (3-6 mg/L). Median trough at 24h for 43 Kg (dashed line): 3.78 mg/L, 70 Kg (solid line): 4.27 mg/L and 100 Kg (dotted line): 4.64 mg/L (p>0.05).