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Systematic Review and Meta-analysis of the Pharmacokinetics of Benznidazole in the Treatment of Chagas Disease

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Chagas disease is a neglected parasitic illness affecting approximately 8 million people, predominantly in Latin America. Benznidazole is the drug of choice for treatment, although its availability has been limited. A paucity of knowledge of the pharmacokinetic properties of this drug has contributed to its limited availability in several jurisdictions. The objective of this study was to conduct a systematic literature review and a Bayesian meta-analysis of pharmacokinetic studies to improve estimates of the basic pharmacokinetic properties of benznidazole. A systematic search of the Embase, Medline, LILACS, and SciELO (Scientific Electronic Library Online) databases was conducted. Eligible studies reported patient-level data from single-100-mg-dose pharmacokinetic evaluations of benznidazole in adults or otherwise provided data relevant to the estimation of pharmacokinetic parameters which could be derived from such studies. A Bayesian hierarchical model was used for analysis. Secondary data (i.e., data from studies that did not include patient-level, single-100-mg-dose data) were used for the generation of empirical priors for the Bayesian analysis. The systematic search identified nine studies for inclusion. Nine pharmacokinetic parameters were estimated, including the area under the concentration-time curve (AUC), the maximum concentration of drug in plasma (C_{max}), the time to C_{max}, the elimination rate constant (k_{el}), the absorption rate constant (K_a), the absorption and elimination half-lives, the apparent oral clearance, and the apparent oral volume of distribution. The results showed consistency across studies. AUC and C_{max} were 51.31 mg · h/liter (95% credible interval [CrI], 45.01, 60.28 mg · h/liter) and 2.19 mg/liter (95% CrI, 2.06, 2.33 mg/liter), respectively. K_a and k_{el} were 1.16 h^{-1} (95% CrI, 0.59, 1.76 h^{-1}) and 0.052 h^{-1} (95% CrI, 0.045, 0.059 h^{-1}), respectively, with the corresponding absorption and elimination half-lives being 0.60 h (95% CrI, 0.38, 1.11 h) and 13.27 h (95% CrI, 11.79, 15.42 h), respectively. The oral clearance and volume of distribution were 2.04 liters/h (95% CrI, 1.77, 2.32 liters/h) and 39.19 liters (95% CrI, 36.58, 42.17 liters), respectively. A Bayesian meta-analysis was used to improve the estimates of the standard pharmacokinetic parameters of benznidazole. These data can inform clinicians and policy makers as access to this drug increases.

Chagas disease, also known as American trypanosomiasis, is a parasitic illness affecting approximately 8 million people worldwide, with most cases being found in continental Latin America (1), although it is increasingly recognized in developed countries outside the traditional area of endemicity for vectorial transmission, due to migration and vertical transmission to the offspring of infected migrant mothers. Chagas disease is primarily transmitted by exposure to the feces of infected triatomine bugs, also known as "kissing bugs." Infection can also occur through means such as mother-to-child transmission, transfusion from the blood of an infected individual, or organ transplantation from an infected donor or can be foodborne. During the acute phase of infection, patients tend to have a variety of symptoms ranging from skin lesions and a swelling eyelid to flu-like symptoms, including fever, headache, and muscle pain. Chronic Chagas disease can lead to more critical injuries, with up to 30% of patients suffering from cardiac disorders and up to 10% suffering from digestive or neurological symptoms. As injury to the cardiovascular system progresses, Chagas disease can lead to sudden death or heart failure, caused by progressive destruction of the heart muscle and its nervous system (2, 3).

Two drugs are currently used for the treatment of Chagas disease and have been shown to be very effective if they are used early in the disease process. Both benznidazole, a nitroimidazole derivative, and nifurtimox, a nitrofuran, act on the parasite through the formation of free radicals and/or electrophilic metabolites. Of these two drugs, benznidazole is the preferred agent because of a lower incidence of side effects (4–6). Recent evidence suggests that benznidazole is also effective in the chronic phase of Chagas disease, although in a randomized clinical trial, treatment significantly reduced the rate of detection of circulating parasites but did not reduce clinical progression to cardiac disorders (7, 8). The availability of both treatments has been limited, however, and Doctors Without Borders/Médecins sans Frontières reported major shortages of benznidazole in 2011, as the primary manufacturer, Hoffmann-La Roche, suspended production and transferred the technology and license to Lafepe Labs in Brazil in 2003

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TABLE 1 Scope of review in terms of PIOS criteria[a]

Criterion	Scope of review
Population	Healthy population and patients with Chagas disease
Intervention	Benznidazole
Outcomes	In adults receiving a single 100-mg dose of benznidazole, the following outcomes were evaluated: C_{max} T_{max} AUC_{0-t} and $AUC_{0-\infty}$ CL/*F* *V*/*F* k_{el} and elimination $t_{1/2}$ K_a and absorption $t_{1/2}$
Study design	All trial types with PK evidence able to inform any of the above-mentioned outcomes

[a] PIOS, population, interventions, outcomes, and study design; AUC_{0-t}, AUC from time zero to time *t*; $AUC_{0-\infty}$, AUC from time zero to infinity; $t_{1/2}$, half-life.

(9). Bayer has since renewed production of nifurtimox, while Elea Laboratory in Argentina has been producing benznidazole since 2012. In 2014, Elea started a joint project with Liconsa labs (Chemo Group) in Spain, which is currently under FDA revision process for approval. Recently, Lafepe Labs in Brazil announced that the Brazilian regulatory agency has approved the use of its benznidazole product. In 2011, a 12.5-mg pediatric dosage form (manufactured by Lafepe Labs and DNDi) was registered by the Brazilian Health Surveillance Agency to further improve the treatment of pediatric Chagas disease.

With the inconsistent availability of benznidazole throughout several countries, it is of critical importance that basic pharmacokinetic data be available to both clinicians and policy makers to ensure evidence-informed decision making with regard to the drug approval process. Thus, there is a requirement for a meta-analysis of studies of the pharmacokinetics (PK) of benznidazole with a special focus on the type of population studied (i.e., the type of population according to age, ethnic background, dose, and regimen).

The purpose of this study was to conduct a meta-analysis of the pharmacokinetic studies of benznidazole that have been conducted in an effort to improve estimates of the basic pharmacokinetic properties of benznidazole.

MATERIALS AND METHODS

Systematic literature search. A comprehensive search of the literature was conducted using the Embase and Medline databases and the Latin American databases SciELO (Scientific Electronic Library Online) and LILACS. The Embase and Medline literature search strategies were conducted using the OVID platform. The search was conducted on 4 May 2016, and the search strategy is provided in appendix A in the supplemental material. The scope of the systematic literature review can be broken down into four components: population, interventions, outcomes, and study design (Table 1).

Study selection and data extraction. A study investigator evaluated all abstracts and proceedings that were identified through the literature search to be potentially relevant for the research question. Those considered potentially relevant were evaluated in full (i.e., the full text publication was acquired) to determine final eligibility status. For all eligible studies, data on study characteristics, patient characteristics, and outcomes were extracted in duplicate by two investigators. Any discrepancies observed between the data extracted by the two data extractors were resolved through discussion, and when discrepancies could not be resolved, a third reviewer was consulted. Where measures were available only in graphical format, the software DigitizeIt (DigitizeIt, Braunschweig, Germany) was used, when possible, to extract the relevant data. When individual patient data (IPD) were available, they were extracted preferentially to summary data. The following study characteristics were extracted: author, year, journal/source, number of patients enrolled, study region, drug dose, drug manufacturer, analytical method, and inclusion/exclusion criteria. The following patient characteristics were extracted: age, sex, weight, body mass index, serum creatinine concentration, and creatinine clearance. The following outcomes were extracted: plasma drug concentration according to time and summary parameters when no IPD were provided, including oral clearance (CL), oral volume of distribution (*V*), half-life, maximum concentration in plasma (C_{max}), time to C_{max} (T_{max}), absorption rate constant (K_a), elimination rate constant (k_{el}), and the area under the concentration-time curve (AUC).

Meta-analysis. Traditional meta-analysis uses summary data from different studies, which are often obtained from publications, to estimate the parameters of interest. In this meta-analysis, data from individual patients were synthesized, resulting in a meta-analysis of the IPD. The approach with IPD improves the quality of the data, the analyses, and, subsequently, the reliability of the results. In addition, the information from summary statistics was also integrated into an all-encompassing meta-analysis. Given the complexity of the analysis, a Bayesian approach was favored for its ability to deal with complex hierarchical models.

Analysis. Bayesian methods involve a formal combination of a prior probability distribution (which reflects a prior belief of the possible values

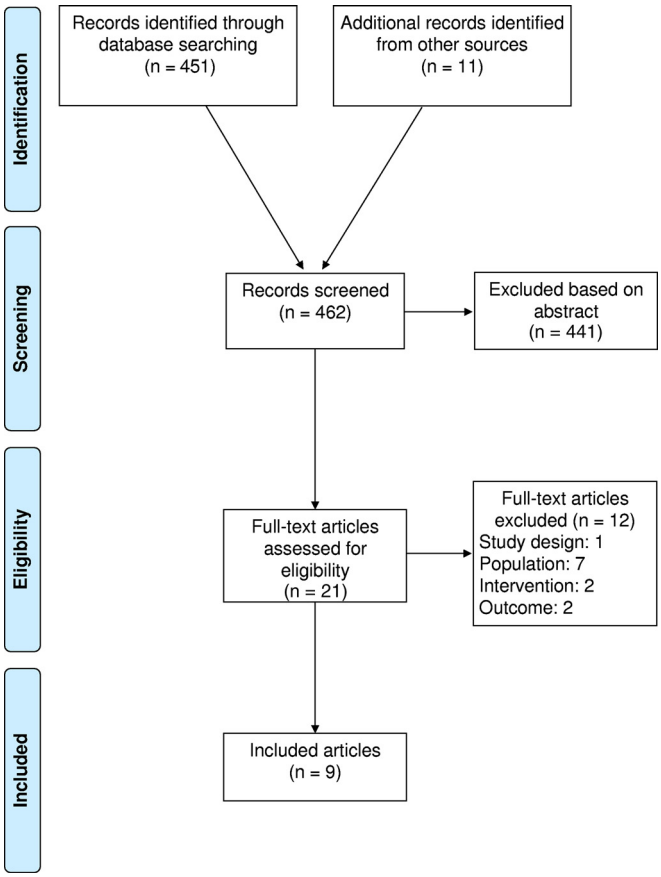

FIG 1 PRISMA (preferred reporting items for systematic reviews and meta-analyses) flow diagram of systematic literature search.

TABLE 3 Final overall model pharmacokinetic parameters[a]

Author(s), yr (reference no.)	AUC (mg · h/liter)	C_{max} (mg/liter)	k_{el} (h^{-1})	$t_{1/2}$[b] (h)	T_{max} (h)	Absorption $t_{1/2}$ (h)	K_a (h^{-1})	V/F (liters)	CL/F (liters/h)
Overall	51.31 (45.01, 60.28)	2.19 (2.06, 2.33)	0.05 (0.04, 0.06)	13.27 (11.79, 15.42)	2.93 (2.57, 3.48)	0.60 (0.38, 1.11)	1.16 (0.59, 1.76)	39.19 (36.58, 42.17)	2.04 (1.77, 2.32)
Bronn, 2015 (17)	50.05 (45.91, 54.34)	2.20 (2.06, 2.34)	0.05 (0.05, 0.06)	12.95 (12.03, 13.95)	2.75 (2.41, 3.17)	0.59 (0.49, 0.72)	1.18 (0.96, 1.41)	39.16 (36.69, 42.04)	2.09 (1.93, 2.29)
Peregrina Lucano, 2004 (20)	54.82 (48.20, 64.40)	2.11 (1.92, 2.29)	0.05 (0.04, 0.06)	14.02 (12.26, 16.66)	3.65 (2.77, 5.27)	0.85 (0.58, 1.44)	0.82 (0.48, 1.20)	39.38 (36.79, 42.55)	1.95 (1.65, 2.23)
Raaflaub and Ziegler, 1979 (22)	49.76 (44.66, 55.38)	2.26 (2.09, 2.44)	0.05 (0.05, 0.06)	12.94 (11.84, 14.28)	2.35 (1.92, 2.93)	0.47 (0.36, 0.64)	1.46 (1.08, 1.92)	39.04 (36.27, 42.01)	2.09 (1.87, 2.33)

[a] Data represent the mean (95% CrI).
[b] $t_{1/2}$, half-life.

TABLE 2 Characteristics of included studies[a]

Author(s), yr (reference no.)	Title	Treatment duration (days)	No. of subjects	Treatment	Analytical method	Drug manufacturer
Raaflaub and Ziegler, 1979 (22)	Single-dose pharamcokinetics of the trypanosomicide benznidazole in man	1	6	Benznidazole at 100 mg (single dose)	Differential pulse polarography	Hoffmann-La Roche
Bronn, 2015 (17)	A study to evaluate the food effect of a new formulation containing 100mg benznidazole. A monocentric, open, randomized, single dose, two-period crossover trial in healthy volunteers	1	18	Benznidazole at 100 mg (single dose)	LC/MS-MS	Laboratorios Liconsa S.A., Spain
Soy et al., 2015 (25)	Population pharmacokinetics of benznidazole in adult patients with Chagas disease	56	49	Benznidazole at 2.5 mg/kg BID	HPLC	Elea Laboratory, Argentina
Raaflaub, 1980 (21)	Multiple-dose kinetics of the trypanosomicide benznidazole in man	25	8	Benznidazole at 3.5 mg/kg BID	Differential pulse polarography	Hoffmann-La Roche
Fernandez et al., 2016 (18)	Pharmacokinetic and pharmacodynamic responses in adult patients with Chagas disease treated with a new formulation of benznidazole	60	6	Benznidazole (various doses)	HPLC	Elea Laboratory, Argentina
Peregrina Lucano, 2004 (20)	Farmacocinetica poblacionalde benznidazole en pacientes Mexicanos con enfermedad de Chagas	1	11	Benznidazole at 100 mg (single dose) + 2.5 mg/kg BID	HPLC	Hoffmann-La Roche
Bournissen, 2013 (19)	E1224 pharmacokinetics report	60	45	Benznidazole at 2.5 mg/kg BID	NR	NR
Roberts et al., 1984 (24)	A phase I study of the combination of benznidazole and CCNU in man	Various	11	Benznidazole at 25 mg/kg	HPLC	Hoffmann-La Roche

[a] The study by Richle and Raaflaub (23) is not reported in this table since the relevant data are already included in ths study by Raaflaub (21). NR, not reported; LC/MS-MS, liquid chromatography-tandem mass spectrometry; HPLC, high-performance liquid chromatography; BID, twice a day.

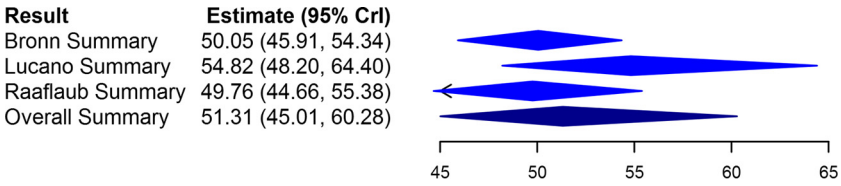

FIG 2 Study-level Forest plot for AUC (in milligram · hours per liter, as indicated on the *x* axis). The midline of each estimate (row) represents the point estimate for the individual study (light blue) and the pooled estimate (dark blue). Each estimate extends to include the lower (left) and upper (right) bounds of the 95% credible interval. The small arrow at the end indicates that the credible interval extends slightly beyond the scale of the *x* axis. Bronn, Lucano, and Raaflaub, the studies of Bronn (17), Pergrina Lucano (20), and Raaflaub and Ziegler (22), respectively.

of the model parameters) with a (likelihood) distribution based on the observed data to obtain a posterior probability distribution of the model parameters (10). The likelihood informs us about the extent to which different values for the parameter of interest are supported by the data. A major advantage of the Bayesian approach is that the method naturally leads to a decision framework (10–12) The posterior distribution can be interpreted in terms of probabilities (e.g., there is an *x* percent probability that treatment A results in a greater response than treatment B); frequentist approaches do not allow such an interpretation (13).

The averaged likelihood is necessary in order for the posterior to be a distribution. By definition, calculation of the averaged likelihood (and, because of that, the posterior distribution) involves integration. This integration can become exorbitant, especially when the parameter of interest is high dimensional. For years, the popularity of Bayesian statistics suffered from the impracticable numerical integrations necessary to obtain the posterior distribution. This changed after the introduction of Markov chain Monte Carlo (MCMC) techniques, which resulted in a rise in popularity of Bayesian statistics because it provides a tool to get around the integration process. The most important and famous MCMC methods include the Gibbs sampler (14) and the Metropolis-Hasting algorithm (15). The Gibbs sampler is based on the characteristic that the multivariate distribution is uniquely determined by its conditional distributions and was used throughout these analyses.

PK. PK is a well-established field in which many different models are used to explain the absorption, distribution, and elimination of a drug within the bloodstream. For this study, a single-compartment model with the following core equation was used:

$$\text{concentration} = \frac{F \times \text{dose} \times K_a}{V \times (K_a - k_{\text{el}})}(e^{-k_{\text{el}} \times \text{time}} - e^{-K_a \times \text{time}}) \quad (1)$$

where K_a is the absorption rate constant, k_{el} is the elimination rate constant, F is bioavailability, and V is the volume of distribution. It turns out that all of the parameters of interest can be expressed as a function of three parameters: K_a, CL, and V. V is the parameter that describes the tendency of a drug to distribute out of the blood into the tissues. It represents the volume of plasma necessary to account for all the drug in the body. The elimination process is defined as the irreversible removal of drugs from the body. The elimination mechanism is best described by the parameter CL. Clearance is the theoretical volume of blood which is effectively cleared of drug per unit of time. The formulas for the remaining parameters are as follows:

$$k_{\text{el}} = \frac{\text{CL}}{V} \quad (2)$$

$$\text{AUC} = \frac{F \times \text{dose} \times K_a}{V \times (K_a - k_{\text{el}}) \times k_{\text{el}}} \quad (3)$$

$$T_{\max} = \frac{1}{(K_a - k_{\text{el}})} \ln\left(\frac{K_a}{k_{\text{el}}}\right) \quad (4)$$

$$C_{\max} = \frac{F \times \text{dose} \times K_a}{V \times (K_a - k_{\text{el}})}(e^{-k_{\text{el}} \times T_{\max}} - e^{-K_a \times T_{\max}}) \quad (5)$$

$$t_{a,1/2} = \frac{\ln(2)}{K_a} \quad (6)$$

$$t_{\text{el},1/2} = \frac{\ln(2)}{k_{\text{el}}} \quad (7)$$

where $t_{a,1/2}$ and $t_{\text{el},1/2}$ are the absorption and elimination half-lives, respectively.

Thus, we used the PK model described in equation 1 as the basis for the hierarchical model and derived the parameters in equations 2 to 7 from the model parameters.

Hierarchical modeling. To discuss the modeling, let y_{ijk} be the kth observation from the ith individual from the jth study with the corresponding time t_{ijk}. The 3 by 1 vector of pharmacokinetic parameters for individual i in the jth study is given by λ_{ij}. The first stage of the model was specified as

$$p(y_{ijk}|\lambda_{ij}, \tau) = N(f_{ijk}, \tau^{-1} v_{ijk}) \quad (8)$$

where f_{ijk} is the pharmacokinetic model evaluated at time t_{ijk} with the individual PK parameters, equal to λ_{ij}, v_{ijk} is the residual error structure, p is the probability function, N is the normal distribution, and τ is the between-patient heterogeneity.

The second stage of the model was to model at the study level and was specified as

$$p(\lambda_{ij}|\theta_j, \Phi) = \text{MVN}(\theta_j, \Phi) \quad (9)$$

where MVN(·) represents a multivariate normal distribution, θ_j (3 by 1) represents the mean kinetic behavior of the ith individual, and Φ (3 by 3) is the corresponding variance-covariance matrix representing the within-study variance.

The third stage of the hierarchical model represents the population

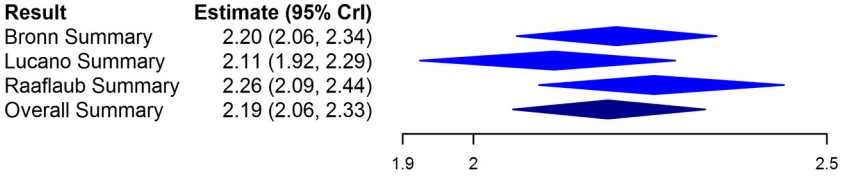

FIG 3 Study-level Forest plot for $C_{\max}$ (in milligrams per liter, as indicated on the *x* axis). The midline of each estimate (row) represents the point estimate for the individual study (light blue) and the pooled estimate (dark blue). Each estimate extends to include the lower (left) and upper (right) bounds of the 95% credible interval.

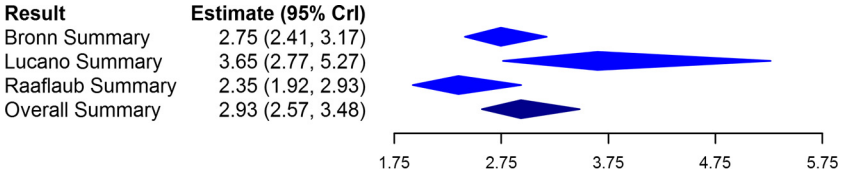

FIG 4 Study-level Forest plot for T_{max} (in hours, as indicated on the *x* axis). The midline of each estimate (row) represents the point estimate for the individual study (light blue) and the pooled estimate (dark blue). Each estimate extends to include the lower (left) and upper (right) bounds of the 95% credible interval.

parameter estimation and was defined by making the following distributional assumptions:

$$p(\theta_i|\mu, \Omega) = \text{MVN}(\mu, \Omega) \quad (10)$$

where μ (3 by 1) is the mean value of the individual mean parameter vector θ_i, and Ω (3 by 3) is the corresponding variance-covariance matrix representing the between-study variance.

The definition of the hierarchical model is completed by the specification of the fourth stage, in which prior densities are assigned to the parameters. In particular, the variance-covariance matrices are defined using a Wishart prior distribution, the population PK parameters are given a multivariate normal prior distribution, and the residual variance factor is defined using an inverse uniform distribution.

In addition to using a hierarchical model to account for the within-individual and study correlation, the model also used an adjustment for whether patients had had food or were fasting. This was accomplished by having a regression adjustment on the absorption rate parameter, such that K_a was replaced by $(K_a - \beta_x)$, where β_x is a metaregression adjustment term used to account for whether a patient is fasting or not, throughout equation 1. It was judged that food but not the volume of distribution or clearance would affect absorption.

In order to integrate the summary statistics from four studies, the information was used to create empirical priors for clearance and volume. In this way, the analysis included a 5th stage by which the information from summary statistics was first integrated and then updated using the four hierarchical stages described above.

The data were analyzed in R (version 3.2.1). The Bayesian analyses were performed using an MCMC method implemented in the JAGS (version 3.4.0) software package (16). A first series of 60,000 iterations from the JAGS sampler was discarded as burn-in, and the inference was based on an additional 100,000 iterations using two chains.

RESULTS

Evidence base. A total of 462 citations were identified through the database search and through a hand search of the literature (Fig. 1). Of these, 441 were excluded at the abstract screening stage. This resulted in the screening of the full text of the articles describing 21 studies. Of these, 12 were excluded: 1 because of an ineligible study design, 7 because the populations studied did not inform the primary analysis of interest (i.e., a single-dose pharmacokinetic analysis), 2 because the intervention of interest was not included, and 2 because the outcome of interest was not included. This resulted in a total of 9 studies that were included in the analysis (17–25). There were no single-dose PK studies of benznidazole in children. The final list of studies included in the analysis is presented in Table 2.

The nine studies included were published or released between 1979 and 2016. One of these studies was a secondary publication of data contained in a prior study, and therefore, the data from that study were not included separately in the final data extraction sheets (23). Three studies contained individual patient-level data from studies of a single benznidazole dose of 100 mg (17, 20, 22). One was a published study (22); one was an unpublished trial report (17); and one was a Ph.D. thesis (20), which also remained unpublished and was obtained from the corresponding university archives with authorization for the purpose of this analysis. Three studies contained limited individual patient-level data from multidose studies (18, 20, 21). Of these, only the study by Raaflaub (21) provided data pertinent to the primary analyses. One additional study contained some further single-dose summary data. This was a study of a single dose of 25 mg/kg in oncology patients (24). A further two studies evaluated benznidazole, using typical therapeutic doses, in a sample of patients with Chagas disease. These studies provided some summary kinetic parameters that were available for incorporation as priors into the final PK model (19, 25).

Pharmacokinetic parameters. Nine pharmacokinetic parameters were estimated at the individual level and study level and as an overall estimate that included the use of empirical priors, when available. The study-level and overall adjusted data along with 95% credible intervals are presented in Table 3. The study-level and overall adjusted data along with 90% credible intervals (CrIs) are presented in appendix B in the supplemental material.

Area under the curve. The overall AUC for the final 100-mg-dose model, including all the available data, was 51.31 mg · h/liter (95% CrI, 45.01, 60.28 mg · h/liter). Only three studies informed this parameter (17, 20, 22). The consistency between studies was excellent, with little heterogeneity from visual assessment of the Forest plot (Fig. 2). The Forest plot with the corresponding 90% credible intervals is shown in appendix B in the supplemental

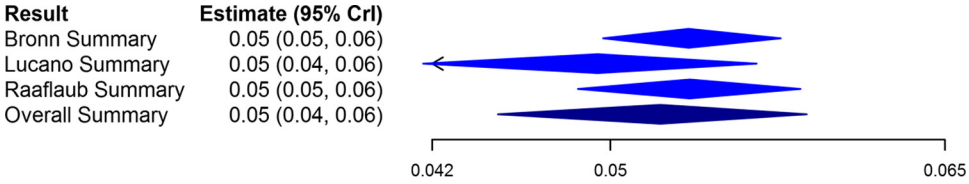

FIG 5 Study-level Forest plot for k_{el} (in hours^{-1}, as indicated on the *x* axis). The midline of each estimate (row) represents the point estimate for the individual study (light blue) and the pooled estimate (dark blue). Each estimate extends to include the lower (left) and upper (right) bounds of the 95% credible interval. The small arrow at the end indicates that the credible interval extends slightly beyond the scale of the *x* axis.

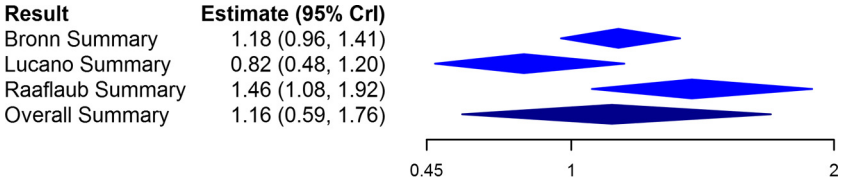

FIG 6 Study-level Forest plot for K_a (in hours^{-1}, as indicated on the *x* axis). The midline of each estimate (row) represents the point estimate for the individual study (light blue) and the pooled estimate (dark blue). Each estimate extends to include the lower (left) and upper (right) bounds of the 95% credible interval.

material. All 90% credible intervals of the individual studies fell within 80% and 125% of the overall estimate, suggesting acceptable heterogeneity. The study presented in the thesis by Peregrina Lucano (20) deviated the most from the overall parameter estimate, but it deviated by only approximately 6%.

Maximum plasma concentration. The overall C_{max} for the final 100-mg-dose model with all the available data was 2.19 mg/liter (95% CrI, 2.06, 2.33 mg/liter). The same three studies that informed the AUC informed this parameter (17, 20, 22). Although the variability in C_{max} between studies was higher than that for AUC, the 90% and the 95% credible limits of each of the individual studies remained between 80% and 125% of the overall estimate. Figure 3 shows the Forest plot for C_{max}, and it can be observed that heterogeneity was minimal, with the point estimates being contained in all the credible intervals.

Time to maximum plasma concentration. The overall calculated T_{max} was 2.93 h (95% CrI, 2.57, 3.48 h). As with AUC and C_{max}, only the three primary studies informed the analysis of this parameter (17, 20, 22). T_{max} was more heterogeneous between studies than either AUC or C_{max}, but overall, the variability was consistent with the degree of variability seen within studies (Fig. 4). The variability in T_{max} was primarily associated with the absorption rate constant (K_a), which in turn was affected by a variety of factors, including the formulation administered and patient factors, such as gastric emptying and, potentially, food effects. Given a constant elimination rate constant, as K_a decreases, T_{max} increases. Since K_a is inherently more variable and difficult to measure, there is likely to be a higher degree of variability in parameters such as T_{max} than in C_{max} or AUC.

Elimination rate constant. The elimination rate constant utilized the patient-level data from the three primary studies (17, 20, 22) but was further informed by two additional studies that provided summary (study-level) data that could be incorporated into the Bayesian model as empirical priors (21, 24). The overall elimination rate constant was 0.052 h^{-1} (95% CrI, 0.045, 0.059 h^{-1}). The study-level data from the three primary studies (17, 20, 22) were very consistent with the overall estimates, as visually depicted in Fig. 5. The final estimate of the elimination half-life was 13.27 h (95% CrI, 11.79, 15.42 h).

Absorption rate constant. The absorption rate constant was the parameter with a high degree of both within-study and between-study heterogeneity (Fig. 6; see also appendix B in the supplemental material). This was reflected in the wide credible intervals in the study-level and overall estimates. The overall estimate was 1.16 h^{-1} (95% CrI, 0.59, 1.76 h^{-1}), with a resulting absorption half-life of 0.60 h (95% CrI, 0.38, 1.11 h). The data from the study presented in the thesis by Peregrina Lucano (20) differed the most from the data from the two other studies.

Apparent volume of distribution. The apparent volume of distribution (V/F) was estimated, and the overall results were remarkably consistent with the estimated results (Fig. 7). Although summary data from two additional studies (19, 25) were used to derive an empirical prior, the results remained consistent. The overall V/F was 39.19 liters (95% CrI, 36.58, 42.17 liters).

Apparent oral clearance. The apparent oral clearance was also estimated utilizing empirical priors for two studies (19, 25). The overall clearance (CL/F) was estimated to be 2.04 liters/h (95% CrI, 1.77, 2.32 liters/h), fitting in well with the results from the three primary studies (17, 20, 22), in which the apparent oral clearances ranged from 1.95 to 2.10 liters/h. Figure 8 shows the study-level Forest plot for clearance, with the results being consistent with each other and the overall estimate.

DISCUSSION

This is the first meta-analysis of pharmacokinetic studies of benznidazole. Using a Bayesian meta-analytic framework, all pharmacokinetic data relevant to the parameters of interest from PK studies of a single benznidazole dose of 100 mg in adults were utilized, thereby producing estimates better than those that could otherwise be derived using a typical frequentist framework. The primary oral PK parameters of interest, including AUC, C_{max}, T_{max}, k_{el}, V/F, and CL/F, showed remarkable consistency between the three primary studies providing patient-level data (17, 20, 22). Although at the individual level there was significant heterogeneity (i.e., within-study heterogeneity), the between-study heterogeneity was modest, suggesting that each study was estimating the population parameter reasonably well and further suggesting that the use of the meta-analytic technique to combine data is well

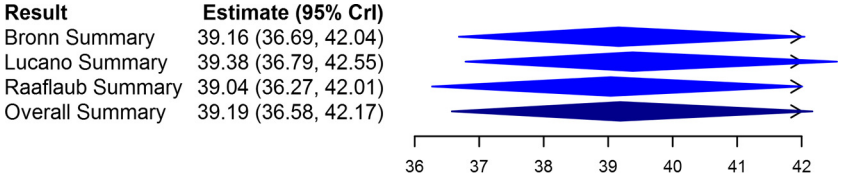

FIG 7 Study-level Forest plot for V/F (in liters, as indicated on the *x* axis). The midline of each estimate (row) represents the point estimate for the individual study (light blue) and the pooled estimate (dark blue). Each estimate extends to include the lower (left) and upper (right) bounds of the 95% credible interval. The small arrows at the ends of the estimates indicate that the credible interval extends slightly beyond the scale of the *x* axis.

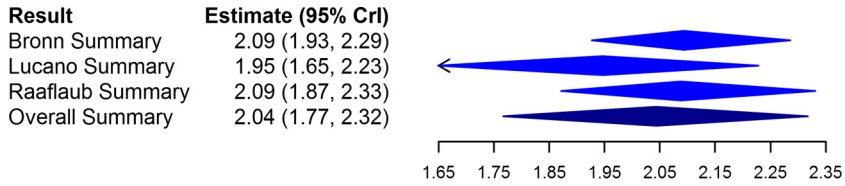

FIG 8 Study-level Forest plot for CL/*F* (in liters per hour, as indicated on the *x* axis). The midline of each estimate (row) represents the point estimate for the individual study (light blue) and the pooled estimate (dark blue). Each estimate extends to include the lower (left) and upper (right) bounds of the 95% credible interval. The small arrow at the end indicates that the credible interval extends slightly beyond the scale of the *x* axis.

justified. When available, additional data from multidose studies and other single-dose studies were used to provide empirical priors to further strengthen the final parameter estimates. These data did not substantially change any parameter estimates, further strengthening the reliability of these results.

An important consideration in meta-analysis is between-study heterogeneity. The degree to which heterogeneity influences the interpretation of results is often subjective and has been widely debated (26). Because few meta-analyses of PK studies have been conducted, the interpretation of between-study heterogeneity is even less well established. As the primary objective of this meta-analysis was to improve the estimates of the oral PK parameters derived from single-dose studies, it must be the case that the studies included in the meta-analysis may actually be sufficiently similar to be combinable. The acceptance criteria for bioequivalence by the Food and Drug Administration is that the 90% confidence intervals of the mean AUC and C_{max} for the test formulation be within 80% and 125% of the values for the reference formulation (27). By taking the reference formulation as the combined estimate and the test formulations as the individual studies, the bioavailability criteria would be met with both AUC and C_{max}. Furthermore, heterogeneity, as visually assessed with the Forest plots, showed consistency between the three primary studies (17, 20, 22) for these outcomes, as well as the other pharmacokinetic parameters.

This study is subject to several limitations. First, this study began with the assumption of a one-compartment model with first-order elimination. While not a limitation *per se*, it assumed that prior studies of the pharmacokinetics of benznidazole performed to determine its basic kinetic properties were correct. A careful examination of the individual-level data presented in appendix C in the supplemental material, however, confirms that the model reflects the data well. Second, although a major advantage of this meta-analysis was the use of patient-level data, these data were collected over a period of more than 30 years from various populations in which a variety of formulations and for which different analytic techniques for drug quantification in plasma were used. However, the consistency of our results, despite these factors, further strengthens the possibility that subsequent studies on the pharmacokinetic characteristics of benznidazole would produce similar parameter estimates.

The treatment of Chagas disease, a paradigmatic case of a neglected tropical disease, suffers from the lack of resources at both the research and implementation levels; therefore, new developments are scarce, and there are plenty of unsolved aspects of the currently available treatments. In view of these limitations, efforts to find solutions to some of these uncertainties through innovative validated analytic methods like this meta-analysis help in the process of the acquisition of knowledge about drugs like benznidazole, around which clear clinical benefits have been observed in certain situations, like acute infections and vertical transmission, but not in others (28).

While this meta-analysis addresses the single-dose pharmacokinetics of benznidazole in adults, these methods could also be applied to both existing pediatric data and multidose data. The advantage of our Bayesian approach was its incorporation of empirical priors into the final analysis. Using this approach, multidose IPD, such as those presented by Raaflaub (21), along with data from other population-based PK studies, could be analyzed. Furthermore, utilizing the single-dose data derived in this meta-analysis, models could be further improved by the incorporation of relevant PK parameters not derived from multidose studies.

In conclusion, this meta-analysis of pharmacokinetic studies has provided improved estimates of the pharmacokinetic parameters under fasting conditions for a single 100-mg dose of benznidazole in adults. The overall results reflect the individual studies from which they were derived. These summary parameters can be used by clinicians and policy makers as the treatment of Chagas disease is scaled up throughout Latin America.

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