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Benznidazole Extended-Release Tablets for Improved Treatment of Chagas Disease: Preclinical Pharmacokinetic Study

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Benznidazole (BNZ) is the first-line drug for the treatment of Chagas disease. The drug is available in the form of immediate-release tablets for 100-mg (adult) and 12.5-mg (pediatric) doses. The drug is administered two or three times daily for 60 days. The high frequency of daily administrations and the long period of treatment are factors that significantly contribute to the abandonment of therapy, affecting therapeutic success. Accordingly, this study aimed to evaluate the preclinical pharmacokinetics of BNZ administered as extended-release tablets (200-mg dose) formulated with different types of polymers (hydroxypropyl methylcellulose K4M and K100M), compared to the tablets currently available. The studies were conducted with rabbits, and BNZ quantification was performed in plasma and urine by ultraperformance liquid chromatography methods previously validated. The bioavailability of BNZ was adequate in the administration of extended-release tablets; however, with the administration of the pediatric tablet, the bioavailability was lower than with other tablets, which showed that the clinical use of this formulation should be monitored. The pharmacokinetic parameters demonstrated that the extended-release tablets prolonged drug release from the pharmaceutical matrix and provided an increase in the maintenance of the drug concentration *in vivo*, which would allow the frequency of administration to be reduced. Thus, a relative bioavailability study in humans will be planned for implementation of a new product for the treatment of Chagas disease.

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*. The parasite is transmitted by hemipteran insects of the subfamily Triatominae (Reduviidae) when a person comes in contact with its contaminated feces/urine through either a scratch in the skin (including the bite), the eyes, or mouth (1, 2). The transmission can also occur by the oral route through contaminated food (3, 4), congenital transmission (5), organ transplantation (6), transfusion of infected blood (7), and laboratory accidents (8).

Chagas disease manifests in two phases: acute and chronic. The acute phase lasts for 2 months with a high parasitemia and may be oligosymptomatic or asymptomatic (9). In the chronic phase, the parasites are hidden in target tissues, especially cardiac tissue and muscles of the digestive system (10). Chagas disease may be more severe in children <5 years of age, immunosuppressed patients (mainly with HIV/AIDS), the elderly, and individuals infected with a large number of parasites (9, 11).

Recently, the World Health Organization (WHO) updated the epidemiological data on Chagas disease from the 21 Latin American countries. This report estimated that 5,742,167 people were infected with *T. cruzi* in the region where it is considered endemic. The 3 countries with the largest estimated numbers of infected people were Argentina, Brazil, and Mexico (1,505,235; 1,156,821, and 876,458, respectively) (12). Due to the immigration of infected persons, cases of the disease have been registered in North America (13), Australia, New Zealand (14), Japan, and Europe (15).

Chagas is a neglected disease due to its pattern of affecting poor communities in large regions, which have little political priority, leading to barriers to control, poor investment in treatment and diagnosis, morbidity, and consequent mortality (16). Only two drugs are available for Chagas disease treatment: nifurtimox and benznidazole (BNZ). BNZ is the only drug widely available (17), and it is highly effective in the acute phase, while in the chronic phase, it is recommended to reduce cardiac symptoms (18). Moreover, BNZ treatment is recommended in congenital cases and reactive infections among all children and for patients up to 18 years old in the indeterminate chronic phase. The BNZ posology (i.e., dosage system) for patients >12 years old is 5 to 7 mg/kg body weight/day, and that for patients <12 years old is 5 to 7.5 mg/kg body weight/day, divided into 2 to 3 daily doses, for 60 days (19). Until 2011, BNZ was only available as a 100-mg tablet, manufactured by Brazilian Pharmaceutical Laboratory of the State of Pernambuco (LAFEPE). Between 2011 and 2012, a 12.5-mg pediatric tablet was developed through the efforts of the Drugs for Neglected Diseases Initiative (DNDi) and LAFEPE. This new pediatric form aimed to minimize the risk of continued use of extemporaneous formulations of tablets for the pediatric population and to avoid imprecise drug administration (20). The 12.5-mg pediatric tablet was registered in Brazil (Brazilian Health Surveillance Agency, ANVISA) in 2011 and is currently present on the

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WHO Model List of Essential Medicines for Children (21). Thus, the treatment of children would be more accurate and safe, resulting in improved treatment of Chagas disease in pediatric patients.

Along the same line, the Technology Laboratory of Medicine of the Federal University of Pernambuco (Recife, Brazil) developed extended-release tablets of BNZ to improve treatment compliance by reducing the number of daily administrations and to increase the residence time of the drug in the body. These tablets were developed using hydroxypropyl methylcellulose (HPMC) for modulation of the drug release rate with different degrees of viscosity (K4M and K100M polymers). In *in vitro* dissolution assays (unpublished data), the release rates (85% dissolution) were 24 h for tablets composed of HPMC K4M polymer and 72 h for tablets composed of the HPMC K100M polymer, compared to the tablets available on the market (immediate-release system) with a release rate of 60 min. Thus, these new tablets with prolonged release of BNZ showed promising results and prospects for *in vivo* testing.

In this study, we evaluated the preclinical pharmacokinetics (PK) of BNZ administered as the commercially available adult (100-mg) and pediatric (12.5-mg) tablets, two types of extended-release tablets (200 mg), and intravenously (i.v.) in healthy white rabbits. In addition, we determined the absolute (*F*) and relative (RBA) bioavailability of the new BNZ tablets. The pharmacokinetic parameters were statistically compared between all groups to determine differences in the kinetic disposition of BNZ from tablets in different administered forms.

MATERIALS AND METHODS

Ethics statement. This study was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences of the São Paulo State University (Araraquara, Brazil) (process 01/2014).

Benznidazole tablets. BNZ immediate-release tablets in the adult (100-mg [lot 13030214]) and pediatric (12.5 mg [lot 13030261]) forms were manufactured and provided by LAFEPE. BNZ extended-release tablets (200 mg) were formulated by the wet granulation process, using as excipients lactose, hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone K30 in hydroalcoholic solution, and magnesium stearate. HPMC polymers with different degrees of viscosity were used: HPMC K4M (4,000 mPa · s in 2% aqueous solution at 20°C) and HPMC K100M (100,000 mPa · s in 2% aqueous solution at 20°C). All quality control testing, characterization of these formulations, and dissolution assays (data not shown) were previously performed by the Laboratory of Medicine Technology of the Federal University of Pernambuco.

Benznidazole administration. Oral administration of the BNZ tablets in rabbits was performed using a pill dispenser with a fixed volume of water (10 ml) in the initial portion of the esophagus. The tablets were administered in full form (not broken) and single dose.

In i.v. administration, BNZ (Sigma-Aldrich, lot MKBL3727V) was dissolved in dimethyl sulfoxide (DMSO). The DMSO volume used (0.3 ml) did not exceed the limit suggested by Neervannan (22) for occurrence of toxic effects. The BNZ solution was administered in the marginal auricular vein of the rabbits in a single 10-mg dose.

Experimental animals. Thirty-five healthy male New Zealand White rabbits (weighing 2.5 to 3.5 kg) were used. The animals were randomly divided into five groups ($n = 7$ in each group) and individually housed in metabolic cages with free access to food and water. Before administration, the rabbits were fasted for 12 h. Food was offered to the animals 4 h after oral administration to prevent food interactions.

The blood samples (100 μl) were collected in heparinized tubes at predetermined intervals for each group. For the pediatric dosage form (12.5-mg tablet), the blood collection times were 0.25 to 6.0 h postdose. For the adult dosage form (100-mg tablet), the blood collection times were 0.25 to 24 h postdose. For extended-release dosage forms (ER-K4M and ER-K100M [200-mg tablets]), the blood collection times were 0.50 to 36 h postdose. With i.v. administration (10 mg), the blood collection times were 0.25 to 8.0 h postdose.

Heparinized blood (50 μl) was separated by centrifugation at 6,000 × *g* for 5 min, and plasma was stored (at −20°C) until chromatographic analysis.

Urine samples were collected using metabolic cages. The urine volume was measured for each collection period (6 h), and aliquots of urine (500 μl) were filtered through nitrocellulose filters and stored (−20°C) until chromatographic analysis.

Plasma analysis. A Waters Acquity ultra-high-performance liquid chromatography (UHPLC) system equipped with a UV-visible (UV-Vis) detector was employed. The chromatographic separation was performed on an Acquity HSS SB C_{18} column (2.1 by 100 mm, 1.8 μm) preceded by an Acquity HSS VanGuard (2.1 by 5 mm; 1.8 μm) guard column at 45°C. The mobile phase was a 65:35 (vol/vol) water-acetonitrile mixture, and elution was in the isocratic mode, with detection at 324 nm. A flow rate of 0.55 ml/min was used, and 1 μl of sample was injected into the chromatographic system. The run time was 2 min.

Prior to the chromatographic analysis, all samples were treated according to the following procedure. Fifty-microliter plasma samples were deproteinized by the addition of 50 μl of acetonitrile (containing 5 μg/ml benzocaine as an internal standard), vortexed for 1 min, and centrifuged at 13,000 × *g* for 10 min. A 50-μl volume of supernatant was withdrawn and filtered with polytetrafluoroethylene (PTFE) syringe filters (0.22 μm, 4 mm) in a maximum recovery vial (Waters). The final extract was stored at 25°C in the sample manager compartment until chromatographic analysis. The linear concentration range was 0.15 to 20 μg/ml. These chromatographic conditions were used in previous studies of BNZ pharmacokinetics in rats (23).

Urine analysis. Urine samples (500 μl) were added 1.5 ml ethyl acetate (containing 50 μg/ml benzocaine as internal standard), vortexed for 30 s, and centrifuged at 2,000 × *g* for 5 min. After phase separation, 1.2 ml organic phase was removed and dried using a sample concentrator under vacuum. The final extract was resuspended in 50 μl acetonitrile, filtered with PTFE syringe filters (0.22 μm, 4 mm) and injected (1 μl) into the chromatographic system. The chromatographic conditions were the same as those used in the analysis of plasma samples. The linear concentration range was 0.078 to 20 μg/ml.

These bioanalytical methods (plasma and urine) were validated based on the U.S. Food and Drug Administration's *Guidance for Industry* (24) and Brazilian Health Surveillance Agency (ANVISA) resolutions 899/2003 (25) and 27/2012 (26).

Pharmacokinetic parameters. The elimination half-life ($t_{1/2}$) was determined by elimination phase of the graph of log plasma concentration versus time. The absorption half-life ($t_{1/2a}$) was determined by the method of residuals. The elimination (k_{el}) and absorption (k_a) constants were calculated by the formula $0.693/t_{1/2}$ or $t_{1/2a}$. k_a was used to calculate the mean absorption time (MAT) by the formula $1/k_a$. The area under the curve from 0 to the last quantifiable concentration (AUC_{0-t}) was calculated by the trapezoidal method, and the area under the curve from 0 to infinity ($AUC_{0-\infty}$) was calculated by the formula $AUC_{0-t} + (C_n/k_{el})$, where C_n was the last quantifiable plasma BNZ concentration. The area under the moment curve (AUMC) was calculated by the statistical moments method and used to determine the mean residence time (MRT): $MRT = AUMC/AUC_{0-\infty}$. The clearance (Cl) and the distribution volume (V_z) were determined by the equations $Cl = dose/AUC_{0-\infty}$ and $V_z = Cl/k_{el}$, corrected by bioavailability for each oral administration group. The maximum plasma drug concentration (C_{max}) was obtained directly from the experimental data, as was the time of the occurrence of C_{max} (t_{max}). *F* and RBA were evaluated by the equations ($AUC_{0-\infty}$ tablet × $dose_{i.v.}$)/($AUC_{0-\infty}$ i.v. × $dose_{tablet}$) and ($AUC_{0-\infty}$ test × $dose_{reference}$)/($AUC_{0-\infty}$ reference × $dose_{test}$), respectively.

Urine analysis was performed to calculate the fraction of dose excreted

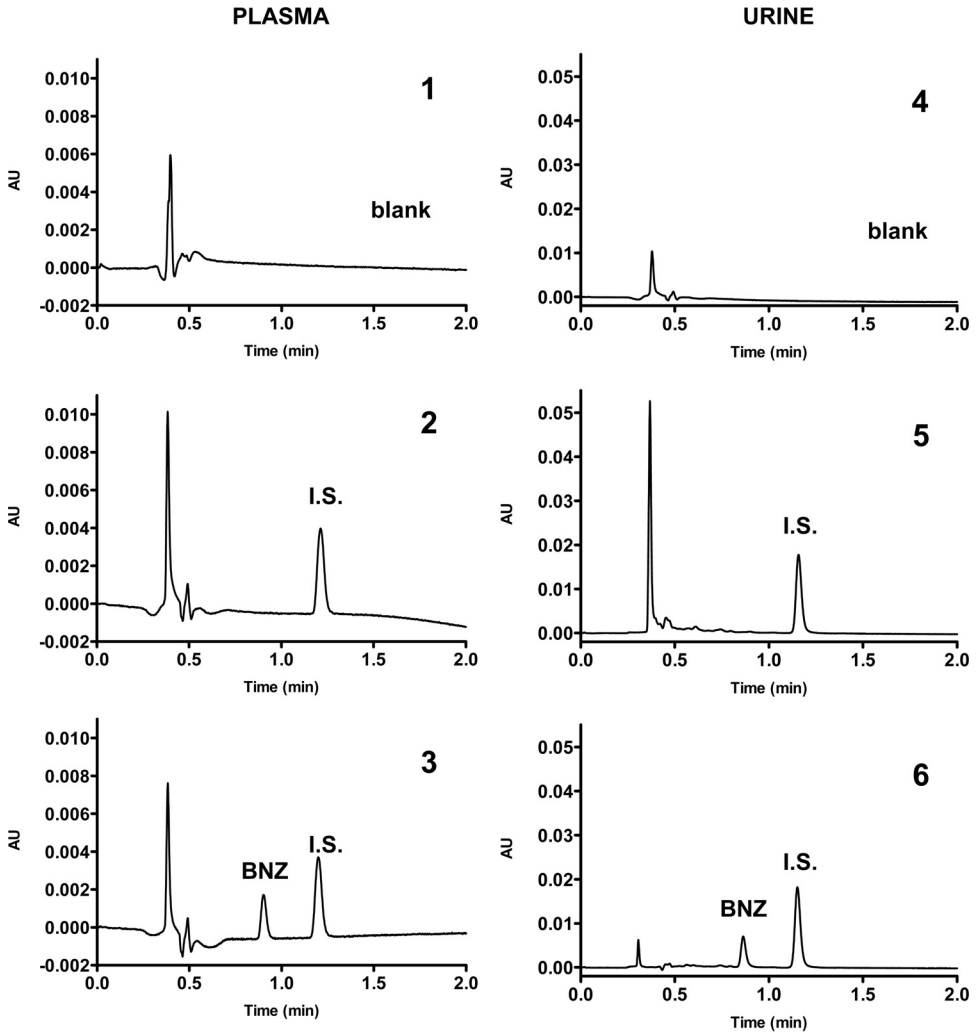

FIG 1 Chromatograms of plasma and urine samples from rabbits showing selectivity of bioanalytical methods for quantification of BNZ. Panels: 1, plasma blank; 2, zero sample of plasma; 3, BNZ at 2.5 μg/ml in plasma; 4, urine blank; 5, zero sample of urine; 6, BNZ at 0.625 μg/ml in urine. I.S., internal standard (benzocaine); AU, absorbance units.

(fe) in urine as unchanged BNZ and the renal clearance (Cl_R) by dividing the total amount of unchanged BNZ excreted in the urine by the AUC_{0-t}.

The formulas described were inserted in Excel software for the calculation of pharmacokinetic parameters, and values were confirmed by PhoenixWinNonlin software.

Statistical analysis. The data for pharmacokinetic parameters are expressed as the mean and 95% confidence interval (95% CI). The groups were compared by the nonparametric Mann-Whitney test (GraphPad Instat software, version 3.06).

RESULTS AND DISCUSSION

Validation of bioanalytical methods. The bioanalytical methods for quantification of BNZ in plasma and urine by UHPLC were validated according to the aforementioned guidelines. The selectivity methods proved adequate by showing that substances in the plasma and urine blank did not interfere with the retention times of BNZ and benzocaine as the internal standard Figure 1 illustrates the chromatograms of plasma (panel 1) and urine (panel 4) blank and zero samples (panels 2 and 5) and plasma (panel 3) and urine (panel 6) samples spiked with BNZ (2.5 μg/ml in plasma and 0.625 μg/ml in urine).

The linearity tests met the acceptance criteria, with precision and accuracy being in accordance with the established limits. The results of precision and accuracy were adequate according to the aforementioned acceptance criteria for analyses of quality controls. The lower limits of quantification (LLOQ) were 0.1562 μg/ml for plasma and 0.039 μg/ml for urine, given the criteria of precision and accuracy. The recovery test also showed precision and accuracy according to the acceptance criteria, demonstrating that sample processing was suitable for the analysis of BNZ in plasma and urine. The stability of BNZ was evaluated under several conditions, including short-term room temperature, long-term frozen, and postprocessed. BNZ was considered stable if the measured analyte concentration of stored samples was between 85 and 115% of the measured concentration of a fresh sample. The confidence limits for the bioanalytical methods for quantification of BNZ in plasma and urine are given in Table 1.

Pharmacokinetic evaluation. The PK profile of BNZ administered as extended-release tablets is shown in Fig. 2 in comparison to the PK profile of the adult immediate-release tablet (Fig. 2A) and the PK profile of BNZ administered as pediatric immediate-

TABLE 1 Parameters of the bioanalytical methods for quantification of BNZ in plasma and urine

Parameter[a]	Result for:	
	Plasma	Urine
Linearity ($n = 10$)		
Concn range, μg/ml	0.1562–20	0.0390–20
Correlation coefficient	$r = 0.9995$	$r = 0.9998$
Equation	$y = 1.5626x - 0.1968$	$y = 0.8445x - 0.0174$
LLOQ, μg/ml	0.1562	0.0390
Precision, RSD		
Intra-assay	8.18	10.1
Interassay	10.3	10.6
Accuracy		
Intra-assay	93.8	97.3
Interassay	101.6	100.2
Recovery, %	65.5	82.0
Stability, RSD/accuracy		
Short term (24 h at room temp)	3.85/94.2	6.5/96.5
Long term (14 days at 20°C)	5.5/107.3	10.0/101.3
Postpreparative (24 h at 25°C)	3.5/92.4	7.4/103.4

[a] LLOQ, lower limit of quantitation; RSD, relative standard deviation.

release tablet in comparison to the PK profile of i.v. administration (Fig. 2B).

The pharmacokinetic parameters for all groups were tabulated, and statistical comparison is shown in Table 2. A one-compartment model was used for the determination of pharmacokinetic parameters of the BNZ tablet groups, and a two-compartment model was used for the i.v. group.

Comparison between the groups and interpretation of pharmacokinetic parameters. There was no statistical difference in C_{max} values between the adult (8.39 μg/ml), ER-K4M (10.26-μg/ml), and ER-K100M (7.15-μg/ml) tablets, although the dose of extended-release tablets (200 mg) was 2-fold the dose of the immediate-release adult tablet (100 mg). It is important to consider this factor in clinical use, because even though the dose was doubled, the C_{max} levels were close in comparison with the immediate-release adult tablet that is already on the market. According to literature data, plasma concentrations equal to or greater than 20 μg/ml in humans increase the risk of adverse effects, mainly dermal manifestations (27).

The Cl_R and fe parameters for adult, ER-K4M, and ER-K100M tablets were higher than those for the pediatric (12.5 mg) and i.v. (10 mg) administrations, which were given in smaller doses. It is possible to infer that the difference in the Cl_R and fe parameters are related to a possible saturation of the biotransformation system and consequent increase in renal excretion and elimination of the drug unchanged in the urine. It is known that BNZ is mainly metabolized by the hepatic cytochrome P450 (CYP450) enzymes (CYP3A4). Studies in HepG2 cells (human hepatocyte cell line) and rats demonstrated that BNZ induces the expression and activity of CYP3A4, P glycoprotein (P-gp), multidrug-resistance-associated protein 2 (Mrp-2), and glutathione *S*-transferase (GST [hepatic and intestinal]) in a concentration-dependent manner (28). The findings of this study raise the hypothesis that in this animal model (rabbits), similar doses of the adult, ER-K4M, and ER-K100M tablets can lead to saturation of the animal biotransformation system and increase renal excretion of unchanged BNZ. For confirmation of this hypothesis, specific research is required with this animal model to identify and describe this phenomenon.

In studies with mice, it was also observed that with the dose of 78 mg/kg body weight, there is saturation of the biotransformation system and thus consequent changes in the pharmacokinetic parameters (29). In humans, it has been reported that there is linearity in the pharmacokinetic parameters in the dose range of 4 to 30 mg/kg in a phase I study (single dose) (30). Studies with patients who received BNZ for 30 days (7 mg/kg/day twice daily) demonstrated that the C_{max} after the first morning dose decreases with time (approximately 20% on average after 25 days of treatment), suggesting an increase in metabolism or excretion of the drug with chronic use (31).

Therefore, the saturation of the biotransformation system by BNZ in humans can be dependent on the frequency of administration and plasma concentration oscillation (high concentration levels). These changes in Cl_R and fe may not occur with the ad-

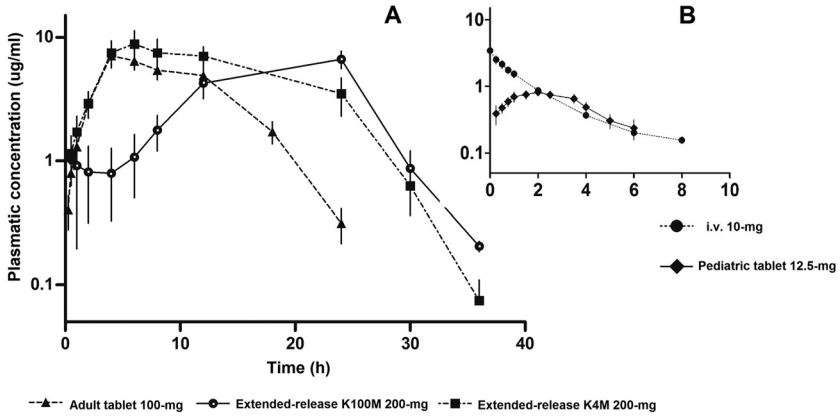

FIG 2 Pharmacokinetic profile after administration of BNZ in tablets and i.v. in rabbits. Values are shown as the mean ± 95% CI. $n = 35$ total ($n = 7$ in each group).

TABLE 2 Pharmacokinetic parameters of oral and intravenous BNZ in rabbits[a]

Unit[b]	Result for BNZ administration, mean (95% CI)[c]				
	Immediate-release tablet			Extended-release tablet[d]	
	Adult (100 mg)	Pediatric (12.5 mg)	i.v. (10 mg)	ER-K4M (200 mg)	ER-K100M (200 mg)
k_{el}, h^{-1}	0.1955 (0.1283–0.2628)	0.4351 (0.3366–0.5335)*	0.2792 (0.1562–0.4023)	0.2076 (0.1231–0.2921)†	0.2309 (0.1464–0.3155)†
$t_{1/2}$, h	4.02 (2.50–5.53)	1.66 (1.34–1.99)*	3.07 (1.65–4.50)	3.92 (2.45–5.41)†	3.56 (1.96–5.17)†
k_a, h^{-1}	0.3476 (0.2971–0.3982)	1.1472 (0.8660–1.4280)*		0.3277 (0.2463–0.4091)†	0.2468 (0.1736–0.3201)*,†
$t_{1/2a}$, h	2.03 (1.73–2.35)	0.63 (0.51–0.75)*		2.24 (1.70–2.79)†	3.19 (1.83–4.54)*,†
MRT, h	9.64 (7.88–11.42)	3.41 (3.03–3.80)*	3.3 (2.20–4.40)*	12.8 (9.25–16.34)†,‡	19.1 (16.0–22.3)*,†,‡,§
MAT, h	2.94 (2.49–3.39)	0.91 (0.74–1.09)*		3.24 (2.45–5.41)†	4.60 (2.64–6.56)*,†
t_{max}, h	6.0 (3.4–8.6)	2.3 (1.6–2.9)*		11.1 (2.9–19.4)†	18.8 (12.9–24.8)*,†
C_{max}, μg/ml	8.39 (6.11–10.67)	0.84 (0.62–1.07)*		10.26 (6.81–13.7)†	7.15 (5.00–9.31)†
V_z, ml/kg	3,165 (1,394–4,936)	1,295 (870–1,721)*	2,338 (1,110–3,567)	2,985 (516–5,454)	3,190 (2,078–4,303)†
Cl, ml/h · kg	515 (406–624)	555 (336–775)	511 (390–632)	483 (246–720)	668 (497–838)
Cl_R, ml/h · kg	9.88 (5.63–14.14)	0.46 (0–0.95)*	1.22 (0.05–2.39)*	8.28 (0–17.01)†	15.68 (10.62–20.74)†,‡
fe, %	1.83 (0.96–2.71)	0.08 (0–0.18)*	0.21 (0–0.42)*	1.45 (0.27–2.64)†	2.43 (1.64–3.23)†,‡

[a] Results are shown as means with 95% CIs in parentheses. $n = 35$ rabbits total (n=7 in each group).
[b] k_{el}, elimination constant; $t_{1/2}$, elimination half-life; k_a, absorption constant; $t_{1/2a}$, absorption half-life; MRT, mean residence time; MAT, mean absorption time; C_{max}, maximum plasma concentration; t_{max}, time to C_{max}; V_z, distribution volume; Cl, total clearance; Cl_R, renal clearance; fe, fraction of drug excreted as unchanged in the urine.
[c] Statistical analysis (Mann-Whitney test): *, $P < 0.05$ compared to the adult tablet group; †, $P < 0.05$ compared to the pediatric tablet group; ‡, $P < 0.05$ compared to the i.v. group; §, $P < 0.05$ compared to the ER-K4M group.
[d] ER-K4M, extended-release tablet composed of type K4M HPMC polymer; ER-K100M, extended-release tablet composed of type K100M HPMC polymer.

ministration of extended-release tablets assessed in future human studies.

Mean residence time (MRT), mean absorption time (MAT), and the time of occurrence of C_{max} (t_{max}) of BNZ for ER-K100M administration were statistically greater than those in all other groups, demonstrating that the extended-release system with the HPMC K100M polymer promoted prolonged drug release, causing a greater drug absorption time and higher maintenance of the plasma drug concentration compared to the other tablets tested. These facts can be considered beneficial for Chagas disease treatment, because this type of tablet can contribute to higher antiparasitic activity.

The pediatric tablet showed faster release and promoted faster absorption than the adult tablet, and it also showed no difference compared to i.v. administration, reinforcing the idea that the drug absorption is much faster in the pediatric formulation, as can be observed in the briefer MAT and t_{max} values. These findings are in accordance with the MRT values observed with the pediatric tablet compared to the others. This may be related to the physicochemical characteristics (size, weight, and hardness) of the tablets and the proportion of excipients (20) in each formulation, leading to different ranges of disintegration and dissolution *in vivo*.

The V_z value found for the pediatric tablet was statistically lower than those for the adult and ER-K100M tablets, which may are related to the lower dose in the pediatric tablet. At higher doses, an eventual saturation of the excretion system may occur, as mentioned above, which allows greater distribution of the drug in the body.

The elimination half-life ($t_{1/2}$) for the pediatric tablet (1.66 h) was statistically shorter than those for the other groups, reinforcing that the elimination of the drug was faster with the administration of this dose. The different dosages between the groups can be a factor to be considered, since there can be a biotransformation system saturation at high doses in this animal model.

Oral bioavailability evaluation. Table 3 presents the mean values of oral F and RBA for each tablet. RBA was calculated based on the adult tablet (100 mg) as the reference.

The bioavailability study demonstrated that the adult tablet, which is currently available for Chagas disease treatment, showed excellent oral absolute bioavailability (F), close to 100%. These results are similar to those found in humans, where Raaflaub and Ziegler showed that absorption of the drug occurred completely after the administration of 100-mg tablets (32). On the other hand, the pediatric tablet (12.5 mg) did not show a value close to

TABLE 3 Results from the oral bioavailability study of BNZ tablets[a]

Parameter[b]	Result for BNZ administration				
	Immediate-release tablet			Extended-release tablet	
	Adult[c]	Pediatric	i.v.	ER-K4M	ER-K100M
Dose,[d] mg/kg	42.2 (36,125–48,381)	4.60 (4,324–4,882)	3.07 (2,962–3,177)	62.9 (58,194–67,794)	68.6 (63,534–73,822)
$AUC_{0-\infty}$, μg/ml · h	86.6 (62.4–110.8)	3.6 (2.3–4.8)	6.3 (5.0–7.6)	153.9 (102.5–205.2)	111.0 (77.3–144.7)
F, %	99.8 (63.1–151.9)	37.7 (25.9–51.0)		118.9 (78.5–170.8)	78.8 (36.8–139.4)
RBA, %		37.8 (21.6–61.4)		119.2 (74.0–171.9)	78.9 (59.5–102.8)

[a] Results are shown as means with 95% CIs in parentheses. $n = 35$ rabbits total ($n = 7$ in each group).
[b] $AUC_{0-\infty}$, area under the concentration-time curve extrapolated to infinity; F, oral absolute bioavailability; RBA, oral relative bioavailability.
[c] Used as the reference group in the RBA calculation.
[d] Corrected dose by weight of the animals.

100%. *F* was 37.7%, and RBA was 37.8%, showing that a little more than one-third of the dose administered reached the bloodstream. It is noteworthy that the pediatric tablet is now available for Chagas disease treatment in children, so pharmacokinetic studies should be performed with these patients to evaluate whether *F* in children is enough to achieve clinical effects.

For extended-release tablets, the bioavailability values were good according to bioequivalence guidelines, which state that RBA should be within the range of 80 to 125% (33). The ER-K100M tablet was close to 80% RBA, which may be related to a long drug release time and the fast gastrointestinal motility of rabbits, which may lead to a fraction of the administered dose being excreted via the digestive tract (feces) without absorption. It is known that gastrointestinal motility and intestinal transit are different for rabbits compared to humans (34), and thus the *F* found in this study for this type of tablet may be different when used in clinical trials (human), since the time in the gastrointestinal tract will be longer and will enable longer drug release.

Conclusion. The pharmacokinetic profile of BNZ in the administration of extended-release tablets allowed the selection of the most advantageous formulation for development. The extended-release K100M tablet exhibited a promising reduction in the frequency of drug administration and enabled a longer residence time in the body. Therefore, a clinical study of relative bioavailability will be conducted in the future with the objective of implementing this new formulation in the treatment of Chagas disease.

The bioavailability of the pediatric tablet was low in comparison with those of the other formulations tested. Considering that this is the first pharmacokinetic study of this formulation, its bioavailability indicates that clinical use of this tablet requires the monitoring of pediatric patients regarding efficacy and plasma drug concentrations.

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