3-1-2021

Benznidazole in cerebrospinal fluid: A case series of chagas disease meningoencephalitis in hiv-positive patients

Marisa L. Fernández
Hospital de Infecciosas Francisco Javier Muniz

María E. Marson
Comision de Investigaciones Cientificas - La Plata

Guido E. Mastrantonio
Comision de Investigaciones Cientificas - La Plata

Marcelo A. Corti
Hospital de Infecciosas Francisco Javier Muniz

Ulises Fleitas
Comision de Investigaciones Cientificas - La Plata

See next page for additional authors

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

CLINICAL THERAPEUTICS

Benznidazole in Cerebrospinal Fluid: a Case Series of Chagas Disease Meningoencephalitis in HIV-Positive Patients

Marisa L. Fernández,[a,b] María E. Marson,[c] Guido E. Mastrantonio,[c] Marcelo A. Corti,[a] Ulises Fleitas,[c] Susana C. Lloveras,[a] Nicolas Lista,[a] Maria M. Priarone,[a] Cecilia Domínguez,[a] Facundo Garcia-Bournissen[d]

[a]Hospital de Infecciosas FJ Muñiz, Buenos Aires, Argentina
[b]Instituto Nacional de Parasitología Dr M Fatala Chaben, ANLIS Dr C Malbran, Ministerio de Salud, Buenos Aires, Argentina
[c]UPL (CICpBA-UNLP), Área Toxicología, Facultad de Ciencias Exactas/UNLP/CONICET, La Plata, Argentina
[d]Division of Paediatric Clinical Pharmacology, Department of Paediatrics, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada

ABSTRACT Chagas disease reactivation in HIV-positive people is an opportunistic infection with 79 to 100% mortality. It commonly involves the central nervous system (CNS). Early treatment with trypanocidal drugs such as benznidazole (BNZ) is crucial for this severe manifestation of *Trypanosoma cruzi* infection. However, limited BNZ clinical pharmacology data are available, especially its concentration in the CNS. We report a series of HIV-positive patients undergoing treatment for *T. cruzi* meningoencephalitis, their clinical response, and cerebrospinal fluid (CSF) and plasma BNZ concentrations. Measurements were carried out using leftover samples originally obtained for routine medical care. A high-performance liquid chromatography/tandem mass spectrometry bioanalytical method designed for BNZ plasma measurements was adapted and validated for CSF samples. Six patients were enrolled in this study from 2015 to 2019. A total of 6 CSF and 19 plasma samples were obtained. Only three of the CSF samples had detectable BNZ levels, all under 1 μg/ml. Fifteen plasma samples had detectable BNZ, and 13 were above 2 μg/ml, which is the putative trypanocidal level. We observed BNZ concentrations in human CSF and plasma. CSF BNZ concentrations were low or not measurable in all patients, suggesting that the usual BNZ doses may be suboptimal in HIV-positive patients with *T. cruzi* meningoencephalitis. While drug-drug and drug-disease interactions may be in part responsible, the factors leading to low CSF BNZ levels remain to be studied in detail. These findings highlight the potential of therapeutic drug monitoring in BNZ treatment and suggest that the use of higher doses may be useful for Chagas disease CNS reactivations.

KEYWORDS Chagas disease, *Trypanosoma cruzi*, meningoencephalitis, HIV/AIDS, benznidazole, therapeutic drug monitoring

Chagas disease (CD), or American trypanosomiasis, reactivation is an opportunistic infection in HIV positive patients commonly presenting as meningoencephalitis and/or central nervous system abscesses (called "Chagomas") and leads to nearly 100% mortality in untreated patients (1–4). Diagnosis of CD reactivation is based on *Trypanosoma cruzi* detection in cerebrospinal fluid (CSF), blood or other fluids or in tissues, usually brain biopsy smears (as amastigote nests with acute inflammatory infiltrates) (5, 6). Immediate treatment with trypanocidal agents such as benznidazole (BNZ) or nifurtimox is essential to reduce morbidity and mortality. BNZ is the most commonly used trypanocidal agent and is usually chosen as first-line treatment. However, understanding of BNZ pharmacokinetics (PK) and pharmacodynamics is quite limited, particularly for a drug that was developed almost 50 years ago (7–13). Information on BNZ absorption, metabolism, distribution, and excretion is scarce, preventing accurate

Citation Fernández ML, Marson ME, Mastrantonio GE, Corti MA, Fleitas U, Lloveras SC, Lista N, Priarone MM, Domínguez C, Garcia-Bournissen F. 2021. Benznidazole in cerebrospinal fluid: a case series of Chagas disease meningoencephalitis in HIV-positive patients. Antimicrob Agents Chemother 65: e01922-20. https://doi.org/10.1128/AAC.01922-20.

Address correspondence to Marisa L. Fernández, marisa.fernandez@gmail.com.

Received 12 September 2020
Returned for modification 14 October 2020
Accepted 8 December 2020
Accepted manuscript posted online 23 December 2020
Published 17 February 2021

prediction of drug-drug and drug-disease interactions. To date, there is virtually no published information on plasma and CSF BNZ concentrations, their pharmacokinetics in HIV-seropositive patients, or the potential interactions between BNZ and antiretrovirals or other drugs (14, 15). Therefore, the pharmacotherapy of CD central nervous system (CNS) reactivations is commonly guided by limited empirical knowledge, and thus the degree of uncertainty surrounding optimal BNZ pharmacotherapy for these patients is high.

The aim of this publication is to report clinical data and BNZ measurements in CSF and plasma from a cohort of HIV-positive patients with CD CNS reactivation treated with BNZ.

RESULTS

A total of 6 patients (age range, 39 to 69 years; 2 women) were included in this cohort, all of them had a previous diagnosis of both HIV infection and positive *T. cruzi* serology. All patients had CNS symptoms at presentation, and all were treated during hospital admission for CNS CD reactivation with BNZ (5 to 8 mg/kg/day twice daily for 60 days) when possible (i.e., if patients survived to complete the full treatment). The time between patient admission to our institution and CD treatment was $<$2 days for most patients, except for patient B, who started BNZ after 5 days, and patient E, who started BNZ on day 10 of hospitalization. Four of the six patients died, yielding a high mortality rate despite appropriate treatment, as has commonly been reported for such severe conditions.

Clinical data and outcomes are presented for each patient in Table 1. All patients had low $CD4^+$ lymphocyte counts (range, 7 to 25 cells/mm^3) on admission. Only patient F was receiving antiretroviral treatment (started 2 weeks before admission; the HIV load was 2,240 copies/μl 2 months after admission). The remaining patients were not taking antiretrovirals due to a history of nonadherence to follow-up as HIV-positive outpatients. All patients received multiple medications during hospital stay (Table 1). The two female patients survived after CD reactivation with neurological improvement and followed up as outpatients for 3 years (patient A) and 6 months (patient F). Patient A started highly active antiretroviral therapy during hospitalization, immediately after the improvement of neurological symptoms (i.e., after 20 days of BNZ treatment). Neurotoxoplasmosis could not be completely ruled out in all patients, since none had undergone brain biopsy and *Toxoplasma gondii* PCR was not available in our institution. Due to the severe clinical presentations and the dire consequences of delaying treatment, all patients received empirical simultaneous treatment for CD and toxoplasmosis. Treatment for *T. gondii* encephalitis was performed with either pyrimethamine plus sulfadiazine or clindamycin or with trimethoprim-sulfamethoxazole. BNZ was well tolerated in most patients. Only one patient (patient A) was switched to nifurtimox 10 mg/kg/day after 30 days of BNZ due to severe leukopenia, with good adherence and response to nifurtimox.

The BNZ concentrations obtained from all CSF and plasma samples are presented in Table 2. Half of the patients CSF samples had undetectable BNZ CSF levels. Patients who had detectable BNZ CSF levels had BNZ concentrations of $<$1 μg/ml. The two survivors of this series had detectable BNZ CSF levels (patient A, 0.5 μg/ml; patient F, 0.3 μg/ml). Paired samples of CSF and plasma to calculate BNZ CSF/plasma ratio were available for patient E (who had undetectable BNZ in plasma and CSF paired samples) and patient F with a BNZ CSF/plasma ratio of 0.1.

Plasma BNZ was quantifiable in 15 of 19 samples; from these 15 samples, 13 had BNZ concentrations above 2 μg/ml, which is the putative trypanocidal level (16). A total of 6 plasma samples had BNZ concentrations below the trypanocidal level; 4 were taken 12 h after BNZ administration (i.e., just before the next dose). Parasitemia, as measured by *T. cruzi* PCR on peripheral venous blood, became undetectable in most of the patients during BNZ treatment, except for one patient (patient E), who had undetectable BNZ levels in most samples and detectable *T. cruzi* PCR on day 13 of BNZ treatment.

TABLE 1 Clinical data at admission, therapeutic data, and clinical evolution[a]

Patient ID	Age (yr)	Sex	CD4	Symptoms	Microscopic detection of parasites		Concomitant medications	Clinical evolution
					Blood	CSF		
A	39	F	9	Right-sided hemiparesis	POS	NA	Omeprazole, dexamethasone, pyrimethamine, clindamycin, ganciclovir, folic acid.	Good response; alive up 3 yrs after Chagas disease reactivation
B	50	M	25	Left-sided hemiparesis	NEG	NEG	Ranitidine, dexamethasone, pyrimethamine, sulfadiazine, phenytoin, metoclopramide, amitriptyline, lorazepam.	Patient died after 1 mo of BNZ treatment prior to stereotactic brain biopsy due to an acute abdominal complication
C	65	M	10	Altered state of consciousness	NA	POS	Omeprazole, dexamethasone, trimethoprim-sulfamethoxazole, amphotericin B, ibuprofen, diphenhydramine	Died after 15 days of hospitalization with 4 confirmed opportunistic CNS infections: *Cryptococcus* spp. (Indian ink and culture positive), CMV (RT-PCR positive on CSF) and culture of *M. tuberculosis* positive on CSF.
D	42	M	7	Seizures	NA	POS	Omeprazole, pyrimethamine, sulfadiazine, phenytoin, lorazepam, fluconazole, prednisone	Initial improvement after *T. cruzi* reactivation but died after 1 mo of treatment due to status epilepticus, probably caused by sequelae of brain lesions.
E	69	M	19	Altered state of consciousness	NA	POS	Omeprazole, dexamethasone, pyrimethamine, clindamycin, ciprofloxacin, leucovorin, fentanyl	Poor clinical course; admitted to intensive care unit, requiring mechanic ventilation complicated with ventilator-acquired pneumonia, multiorgan failure and died after 2 wks of BNZ treatment.
F	65	F	16	Right-sided hemiparesis	NEG	POS	Omeprazole, pyrimethamine, clindamycin, trimethoprim-sulfamethoxazole, leucovorin, fluconazole, tenofovir, emtricitabine, lopinavir-ritonavir	Good clinical response; she recovered and was discharged after 1 mo to continue follow-up as an outpatient

[a]CD4, absolute count of $CD4^+$ cells (cells/ mm^3); F, female, M, male, CNS, central nervous system, CSF, cerebrospinal fluid, CMV, cytomegalovirus. NA, not available, POS, positive, NEG, negative.

TABLE 2 BNZ concentration obtained from all CSF and plasma samples[a]

	Plasma BNZ data			CSF BNZ data		
Patient ID	Duration (days) of BNZ treatment	Plasmatic BNZ (μg/ml)	Sampling time (h) after last intake	Duration (days) of BNZ treatment	CSF BNZ (μg/ml)	Sampling time (h) after last intake
A	23	6.5	0.5	NA	NA	NA
	23	11.9	4	22[b]	0.5	7
B	3	8.8	0.5	NA	NA	NA
	10	2.3	12	NA	NA	NA
	10	6.2	3	10	<LOD	0–4*
	17	<LOD	12	NA	NA	NA
	17	5.5	3	NA	NA	NA
C	7	3.2	9.5	NA	NA	NA
	7	5.5	3.3	7	0.8	0–4*
D	36	5.4	2	26[c]	<LOD	1
	36	1.8	12	NA	NA	NA
E	**6**[d]	<LOD	7.3	6	<LOD	7
	6	<LOD	13	NA	NA	NA
	12	<LOD	12	NA	NA	NA
	13	4.6	1.3	NA	NA	NA
	8	1.5	6	NA	NA	NA
F	**2**	2.8	12	NA	NA	NA
	3	3.2	2	NA	NA	NA
	23	2.8	1	23	0.3	1

[a]The limit of detection (LOD) and limit of quantification were, respectively, 0.1 and 0.2 μg/ml for CSF and 0.5 and 1.4 μg/ml for plasma. *, Between 0 and 4 h after BNZ administration (the exact time was not recorded in the chart). CSF, cerebrospinal fluid; NA, not available. Plasmatic and CSF BNZ concentrations are expressed as $\pm$0.1 μg/ml.
[b]CSF was obtained 1 day prior to the plasma sample.
[c]The CSF sample was recovered 10 days prior to plasma sample.
[d]For values in boldface, BNZ was administered using a nasogastric tube due to patient critical clinical status.

DISCUSSION

Management of HIV/AIDS patients with CNS involvement is very complex, requiring the consideration of multiple, potentially overlapping and severe differential diagnoses (e.g., neurotoxoplasmosis, lymphoma, neurotuberculosis, cryptococcosis, and others) (17). Even though CD CNS reactivation is an infrequent opportunistic CNS infection in HIV patients (17, 18), in areas of endemicity, such as Latin America, CD must be included in the differential diagnosis of brain mass lesions or diffuse meningoencephalitis given its dire prognosis and potentially rapid progression. In this series, 4 of 6 patients died; in one of them, patient B, we could not obtain confirmation of CD reactivation (i.e., brain biopsy was not possible), but CD was highly suspected, so empirical BNZ treatment was given. All of the patients in this series were critical patients, and all of them had extremely low CD4 counts and CNS compromise, so CD reactivation, as well as any and all opportunistic diseases (including those confirmed and those suspected that could not be ruled out), could be responsible for this high death rate.

As suggested in previous CD reactivation series in patients with HIV/AIDS, it is plausible that the difficulties in reaching a diagnosis of CD CNS reactivation, leading to delays in implementing specific treatment (e.g., with BNZ), may be responsible for the observed poor outcomes (1–4). Although, in this series, most patients started BNZ up to day 2 of hospitalization, they still had a poor outcome. Alternative explanations for this include the absence of pharmacokinetic and pharmacodynamic BNZ data in HIV patients, making decisions on appropriate dosing, the duration of treatment, and evaluation of therapeutic responses an extremely difficult and imprecise task.

Previously available BNZ CNS concentration data come from only two studies (19, 20). One study evaluated BNZ penetration into the CNS using CNS tumor biopsy specimens from 17 patients enrolled in a phase 2 clinical trial looking into BNZ treatment as adjuvant chemotherapy. The tumor/mean plasma BNZ ratio was estimated to be >0.46 for all patients, meaning that BNZ CNS concentrations reached nearly 50% of those observed in plasma (19). Another publication is a case report of a single immunocompromised renal transplanted patient with CD CNS reactivation in whom, due to poor

clinical CD CNS evolution, the BNZ dose was empirically doubled, and the BNZ levels in CSF and plasma samples 3 h after dosing were 8.3 and 17.2 mg/liter, respectively (i.e., a BNZ CSF/plasma ratio of 0.48) (20).

The only two survivors in our series had detectable BNZ in CSF, albeit at low levels, and one of them was the only patient in our series for whom we were able to estimate BNZ CSF/plasma ratios (i.e., 0.1), which was far below previously published CNS/plasma or CSF/plasma ratios. These novel observations provoke two main questions: (i) why the CSF BNZ measures in HIV/AIDS patients seemed lower than expected and (ii) whether these low BNZ levels can still be effective, as suggested by these two surviving patients. Unfortunately, we can only advance speculative answers to both of these questions, in the hope that future clinical studies will confirm (or reject) our hypothesis. We believe that the low BNZ CNS levels can be explained by the fact that the timing of the samples may have missed BNZ peak in CSF, suggesting a different PK in these patients. Lumbar punctures in the survivors were done 1 and 7 h after the BNZ doses, so the 1-h sampling time could have been before BNZ peak concentration, and the 7-h sampling time could have captured the trough concentration in a patient with altered PK (e.g., faster clearance, larger volume of distribution, and reduced absorption). However, even if the CSF samples were not taken at the BNZ peak concentration, we would have still expected higher CSF BNZ levels than observed. In the only patient for whom we could calculate BNZ CSF/plasma ratio, that ratio was almost five times lower than those reported in previous publications (19, 20). We believe that the low BNZ CNS concentrations observed could also be due to reasons other than suboptimal sampling timing, including a limited absorption of the drug from the gastrointestinal tract due to the acute and severe clinical picture. Further potential explanations include drug-drug interactions (e.g., all patients were on multiple medications, whose potential for interactions with BNZ have not been formally evaluated to date), and physiological alterations associated with the septic or clinical state of the patients, which could interfere with BNZ CNS access due to altered BNZ pharmacokinetics. The second issue to take into consideration in the context of the survivors is that the BNZ parasite inhibitory concentration may be lower than expected or that BNZ could act via a maximum concentration mechanism with extended postantiparasitic effects (similar to the postantibiotic effect of some antibacterial drugs), affecting parasite growth even after BNZ levels decrease below the putative trypanocidal threshold. The efficacy of lower concentrations of BNZ has been proposed by some preclinical and clinical assays (9, 10, 21, 22).

Patient E was the most critical patient in this series since he was under mechanical ventilation due to a depressed level of consciousness. Unfortunately, most of his samples (plasma and CSF) had no measurable BNZ. We were really concerned about these new data, so we looked into what may have interfered with BNZ. We initially inferred that the reason for the undetectable BNZ could be due to the administration route, through nasogastric tube, since there is no intravenous BNZ available; however, patient F received BNZ through a tube, too, and had normal plasma BNZ levels. The critical clinical condition of patient E could have led to limited gastrointestinal absorption, modified drug volumes of distribution, or the polypharmacy that could lead to drug interactions. In this setting, it is not hard to envision that BNZ therapeutic drug monitoring could yield useful information to better guide dosing decisions, especially in these patients.

The CD World Health Organization expert committee recommended, in 2002, BNZ doses of 25 mg/kg/day for CD acute meningoencephalitis; however, this recommendation was done empirically without published or personal communication data. These high doses did not have any safety data either (23). To date, there is only one case report with safety information on high BNZ doses (15 mg/kg/day) that were well tolerated (20). Otherwise, studies on BNZ doses and schedules from 1979 showed greater and more frequent adverse events with doses between 7 and 10 mg/kg/day than with 5 mg/kg/day (7). Consistent with previous data, we observed BNZ CNS concentrations that were significantly lower than those in plasma and also lower than previously observed (19, 20). This observation raises concerns of insufficient BNZ exposure at current doses in *T. cruzi* CNS reactivation in HIV-positive patients. CD CNS reactivation patients may therefore require higher

doses of trypanocidal drugs such as BNZ, or perhaps alternative treatment schedules that may increase the CNS exposure and improve the likelihood of therapeutic response. If BNZ dosing is increased in these patients, particularly given the risk of therapeutic failure, close adverse event monitoring is advisable.

An important limitation of our study is that the samples were obtained for clinical (i.e., not pharmacokinetic) reasons, leading to quasirandom sampling times in regard to the time of BNZ dose administration. However, as mentioned previously, BNZ pharmacokinetics data are scarce, and there are no accurate data available on its clearance, volume of distribution, or penetration of the blood-brain barrier (with or without CNS inflammation) in these patients. Therefore, we believe that the data presented here, albeit incomplete, should help practitioners decide on BNZ dosing regimens, may establish BNZ measurement in critical patients during early follow-up, and should also prompt further BNZ clinical pharmacology studies in this population, as well as encourage the pharmacological industry to develop an intravenous BNZ formulation.

Conclusion. Chagas disease reactivation in HIV patients is a severe complication frequently associated with fatal outcomes. Given the limited data on pharmacotherapy available in the literature, treatment decisions are guided by scarce data and often involve a significant amount of guesswork. We believe that, in the face of such a dearth of evidence, even limited case series, such as the one presented here, can be useful for clinicians dealing with such difficult cases.

Considering the importance of trypanocidal treatment in CD CNS reactivation, BNZ bioavailability problems should be taken into account, and possibly BNZ measurement in blood should be performed to confirm therapeutic levels are needed for correct clinical treatment approach. It is also clear from our data that more in-depth knowledge on BNZ pharmacology in these patients is urgently needed. Further clinical trials and cohort studies should be encouraged to fill this knowledge gap.

MATERIALS AND METHODS

A retrospective cohort study was carried out with six HIV- and *T. cruzi*-seropositive patients who presented with CNS involvement compatible with CD reactivation. Clinical charts of patients suspected for CNS Chagas reactivation who received BNZ in our institution, the Hospital de Infecciosas FJ Muñiz, Buenos Aires, Argentina, during a 4-year period (i.e., 2015 to 2019) were reviewed to identify patients eligible for this study. The study protocol was approved and informed consent was waived by the Institutional Ethics in Research Committee of the Hospital de Infecciosas FJ Muñiz, Buenos Aires, Argentina (DI-2020-98-GCABA-HIFJM).

Leftover CSF and plasma samples recovered at the Clinical Analysis Division of Hospital de Infecciosas FJ Muñiz were retrieved for BNZ quantitation for all six patients. Clinical and laboratory information were obtained from patient charts, including patient age, gender, origin, clinical signs and symptoms at presentation, and during hospital stay, HIV status (including CD4 lymphocyte counts, relevant microbiology studies, concomitant medication, and clinical follow-up). CSF and plasma samples obtained during treatment were recovered for all patients.

CSF and plasma samples were stored at −20°C until measurement by high-performance liquid chromatography/tandem spectrometry (HPLC-MS/MS) following a method developed by our group with the corresponding validation for its application in the CSF matrix (24). The method designed for BNZ plasma measurements was readapted and validated for CSF, as it is a different matrix sample. For validation procedures, bovine CSF was used as a blank matrix to evaluate calibration curves and validation parameters. Plasma and CSF calibration curves were prepared with known standards. Briefly, after sample extraction, measurements were carried out by HPLC-MS/MS (Shimadzu Nexera HPLC and Sciex QTrap 6500 mass spectrometer) using MRM transition 261/91 in positive ESI mode for BNZ quantitation after chromatographic separation in a C_{18} HPLC column with isocratic methanol-water (65:35 [vol/vol]). Concomitant medications were chromatographically evaluated to ensure that they would not interfere with BNZ detection or quantitation in the analytical method. The limit of detection (LOD) and limit of quantitation (LOQ) for BNZ were 0.1 and 0.2 μg/ml for CSF samples and 0.5 and 1.4 μg/ml for plasma samples, respectively.

This study, including the use of stored leftover samples (originally obtained for clinical purposes), was approved by the Institutional Ethics in Research Committee of the Hospital de Infecciosas FJ Muñiz, Buenos Aires, Argentina (DI-2020-98-GCABA-HIFJM).

ACKNOWLEDGMENTS

We thank all of the personnel from Hospital de Infecciosas FJ Muñiz, especially those from Clinical Analysis Division and the wards where the patients were hospitalized to help us to recover the leftover samples we used in this study. We also thank Sarah

Wongseelashote and Neil Aldersley (Wellington Hospital, Wellington, New Zealand) for reviewing the English language of the manuscript.

This study was supported by Bunge & Born Foundation grant 2017/18 on its 7th contest for the Investigation of Chagas Disease and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

We declare that there are no conflicts of interest.

REFERENCES

1. Cordova E, Boschi A, Ambrosioni J, Cudos C, Corti M. 2008. Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients in Argentina, 1992–2007. Int J Infect Dis 12:587–592. https://doi.org/10.1016/j.ijid.2007.12.007.
2. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. 2020. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. NIH, Bethesda, MD. http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed 6 September 2020.
3. Sztokhamer D, Arias L, Dinerstein E, Gustincic M, Gárgano S. 2010. Reactivation of Chagas Disease (American trypanosomiasis) with brain involvement, in HIV-AIDS patients in Argentina. Actualiz SIDA 68:49–54. (In Spanish.) https://www.huesped.org.ar/wp-content/uploads/2014/09/ASEI-68-49-54.pdf. Accessed 6 September 2020.
4. Almeida EA, Ramos AN, Jr, Correia D, Shikanai-Yasuda MA. 2011. Coinfection *Trypanosoma cruzi*/HIV: systematic review (1980–2010). Rev Soc Bras Med Trop 44:762–770. https://doi.org/10.1590/s0037-86822011000600021.
5. Rocha A, de Meneses AC, da Silva AM, Ferreira MS, Nishioka SA, Burgarelli MK, Almeida E, Turcato Júnior G, Metze K, Lopes ER. 1994. Pathology of patients with Chagas disease and acquired immunodeficiency syndrome. Am J Trop Med Hyg 50:261–268. https://doi.org/10.4269/ajtmh.1994.50.261.
6. Lazo JE, Meneses AC, Rocha A, Frenkel JK, Marquez JO, Chapadeiro E, Lopes ER. 1998. Toxoplasmic and chagasic meningoencephalitis in patients with human immunodeficiency virus infection: anatomopathologic and tomographic differential diagnosis. Rev Soc Bras Med Trop 31:163–171. (In Portuguese.) https://doi.org/10.1590/s0037-86821998000200001.
7. Barclay CA, Cerisola JA, Lugones H, Ledesma O, Lopez-Silva J, Mouzo G. 1978. Aspectos farmacólogicos y resultados terapéuticos del benznidazol en el tratamiendo de la infección chagasica. Pren Med Arg 65:239–244. (In Spanish.)
8. Molina I, Salvador F, Sánchez-Montalvá A, Artaza MA, Moreno R, Perin L, Esquisabel A, Pinto L, Pedraz JL. 2017. Pharmacokinetics of benznidazole in healthy volunteers and implications in future clinical trials. Antimicrob Agents Chemother 61:e01912-16. https://doi.org/10.1128/AAC.01912-16.
9. Altcheh J, Moscatelli G, Mastrantonio G, Moroni S, Giglio N, Marson ME, Ballering G, Bisio M, Koren G, García-Bournissen F. 2014. Population pharmacokinetic study of benznidazole in pediatric Chagas disease suggests efficacy despite lower plasma concentrations than in adults. PLoS Negl Trop Dis 8:e2907. https://doi.org/10.1371/journal.pntd.0002907.
10. Fernández ML, Marson ME, Ramirez JC, Mastrantonio G, Schijman AG, Altcheh J, Riarte AR, Bournissen FG. 2016. Pharmacokinetic and pharmacodynamic responses in adult patients with Chagas disease treated with a new formulation of benznidazole. Mem Inst Oswaldo Cruz 111:218–221. https://doi.org/10.1590/0074-02760150401.
11. García-Bournissen F, Moroni S, Marson ME, Moscatelli G, Mastrantonio G, Bisio M, Cornou L, Ballering G, Altcheh J. 2015. Limited infant exposure to benznidazole through breast milk during maternal treatment for Chagas disease. Arch Dis Child 100:90–94. https://doi.org/10.1136/archdischild-2014-306358.
12. Kratz JM, Garcia Bournissen F, Forsyth CJ, Sosa-Estani S. 2018. Clinical and pharmacological profile of benznidazole for treatment of Chagas disease. Expert Rev Clin Pharmacol 11:943–957. https://doi.org/10.1080/17512433.2018.1509704.
13. Wiens MO, Kanters S, Mills E, Peregrina Lucano AA, Gold S, Ayers D, Ferrero L, Krolewiecki A. 2016. Systematic review and meta-analysis of the pharmacokinetics of benznidazole in the treatment of Chagas disease. Antimicrob Agents Chemother 60:7035–7042. https://doi.org/10.1128/AAC.01567-16.
14. Seden K, Khoo S, Back D, Prevatt N, Lamorde M, Byakika-Kibwika P, Mayito J, Ryan M, Merry C. 2013. Drug-drug interactions between antiretrovirals and drugs used in the management of neglected tropical diseases: important considerations in the WHO 2020 Roadmap and London Declaration on Neglected Tropical Diseases. AIDS 27:675–686. https://doi.org/10.1097/QAD.0b013e32835ca9b4.
15. Rodrıguez-Guardado A, Tuset M, Asensi V, Miro JM. 2011. Human immunodeficiency virus and Chagas disease coinfection treated successfully with benznidazole and a raltegravir-based antiretroviral regimen: a case report. Med Clin (Barc) 137:278–279. https://doi.org/10.1016/j.medcli.2010.06.013.
16. Raaflaub J, Ziegler WH. 1979. Single-dose pharmacokinetics of the trypanosomicide benznidazole in man. Arzneimittelforschung 29:1611–1614.
17. Corti M, Metta H, Villafañe MF, Yampolsky C, Schtirbu R, Sevlever G, Garrido D. 2008. Stereotactic brain biopsy in the diagnosis of focal brain lesions in AIDS. Medicina (Buenos Aires) 68:285–290. (In Spanish.)
18. Livramento JA, Machado LR, Spina-França A. 1989. Cerebrospinal fluid abnormalities in 170 cases of AIDS. Arq Neuropsiquiatr 47:326–331. https://doi.org/10.1590/s0004-282x1989000300013.
19. Roberts JT, Bleehen NM, Lee FY, Workman P, Walton MI. 1984. A phase I study of the combination of benznidazole and CCNU in man. Int J Radiat Oncol Biol Phys 10:1745–1748. https://doi.org/10.1016/0360-3016(84)90541-8.
20. Montero M, Mir M, Sulleiro E, Avalos Esquivel JL, García López E, Molina-Morant D, López Montesinos I, Sorlí L, Veliz Espinosa G, Mounteis Oliva E, Crespo M, Monge I, Horcajada JP, Grau S, Pascual J. 2019. High-dose benznidazole in a 62-year-old Bolivian kidney transplant recipient with Chagas central nervous system involvement. Int J Infect Dis 78:103–106. https://doi.org/10.1016/j.ijid.2018.10.014.
21. Bustamante JM, Craft JM, Crowe BD, Ketchie SA, Tarleton RL. 2014. New, combined, and reduced dosing treatment protocols cure *Trypanosoma cruzi* infection in mice. J Infect Dis 209:150–162. https://doi.org/10.1093/infdis/jit420.
22. Molina-Morant D, Fernández ML, Bosch-Nicolau P, Sulleiro E, Bangher M, Salvador F, Sanchez-Montalva A, Ribeiro ALP, de Paula AMB, Eloi S, Correa-Oliveira R, Villar JC, Sosa-Estani S, Molina I. 2020. Efficacy and safety assessment of different dosage of benznidazol for the treatment of Chagas disease in chronic phase in adults (MULTIBENZ study): study protocol for a multicenter randomized phase II non-inferiority clinical trial. Trials 21:328. https://doi.org/10.1186/s13063-020-4226-2.
23. WHO Expert Committee on the Control of Chagas Disease (2000: Brasilia, Brazil) and World Health Organization. 2002. Control of Chagas disease: second report of the WHO expert committee. World Health Organization, Geneva, Switzerland. https://apps.who.int/iris/handle/10665/42443. Accessed 10 November 2020.
24. Marsón ME, Dana DD, Altcheh J, García-Bournissen F, Mastrantonio G. 2013. Development of UV/HPLC methods for quantitative analysis of benznidazole in human plasma and urine for application in pediatric clinical studies. J Clin Lab Anal 27:384–390. https://doi.org/10.1002/jcla.21615.