Open Label Phase-II Randomized Trial of 3 Regimens of Liposomal Amphotericin B as Induction Therapy for Disseminated Histoplasmosis in AIDS patients

STUDY PROTOCOL

CLINICAL TRIALS REGISTRATION NCT04059770

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STUDY CONCEPT

Background

Histoplasma capsulatum is the etiologic agent of histoplasmosis, a systemic mycosis that is highly endemic in the Americas and also being identified in other parts of the globe [1, 2]. Histoplasmosis may disseminate in immunosuppressed patients, particularly those infected by the HIV. Accordingly, histoplasmosis has been an AIDS-defining infection since 1987 [3]. Disseminated histoplasmosis (DH) is one of the main AIDS-defining infections and a major killer of HIV-infected patients in South and Central America [3-5].

Most AIDS patients with histoplasmosis require high dose of antifungal agents. Even though liposomal amphotericin B (L-AmB) is considered the drug of choice for histoplasmosis in AIDS, as agreed in the Infectious Diseases Society of America guidelines [6], and supported by a randomized clinical trial [7], many patients in Latin America are still treated with high doses of deoxycholate amphotericin B (d-AmB) for periods as long as 2-6 weeks. These regimens are associated with considerable toxicity, and therefore reduced efficacy. Overall mortality in patients using d-AmB in Latin America, where *Histoplasma* antigen detection is rarely available, can be as high as 50% [5]. A better treatment strategy is ultimately needed.

L-AmB has several advantages over d-AmB in the treatment o DH: lower infusion-related toxicity [8], and reduced hematological and renal toxicity [9, 10]. In addition, L-AmB has long half-life and effectively penetrates in tissues such as liver, spleen and the central nervous system [11]. Considering that the antifungal effect of the polyenes depends on peak concentrations, treating patients with a high dose of L-AmB for short periods [instead of standard doses for longer periods] is a promising approach. Such strategy has successfully been studied in antifungal prophylaxis [12, 13], febrile neutropenia [14], leishmaniasis [15], and more recently, cryptococcosis [16]. When used for antifungal prophylaxis in hematology patients, doses of up to 15 mg/kg of L-AmB were given without significant toxicities [17]. Conversely, toxicity has been linked to repeated administrations of L-AmB [12]. Despite

these encouraging data, the strategy of using single high dose of L-AmB has never been tried in histoplasmosis.

Research Justification

DH is an endemic disease that frequently occurs in institutions with no access to modern diagnostic tests and that have limited availability of L-AmB. If a strategy of less frequent, high doses of L-AmB shows activity in DH, this will allow for reducing the cost of treatment in developing countries, in addition to improving safety and tolerability. Based on what has been published so far, safety does not seem to be a concern with high single doses of L-AmB. Results of this study may serve as a basis for a future phase III randomized comparative trial.

Objectives

To determine and compare the activity and safety of three regimens of L-AmB, as induction therapy of DH in AIDS patients, as well as to determine the safety of the three regimens.

Methods

Trial design

This will be a prospective randomized non-comparative multicenter open label trial of induction therapy for DH in AIDS, followed by oral therapy with itraconazole.

The total sample size planned is 99 patients (33 patients per study arm). This sample size considers 10% of dropout.

The study will be conducted in compliance with the declaration of Helsinki, as well as ICH GCP guidelines. Participation in the study will require written consent. The study will be registered at www.clinicaltrials.gov, before trial initiation and patient recruitment.

Participants

We will include adult ($\geq$18 years old) patients infected with the HIV (AIDS) who are diagnosed with DH, by the means of either (i) positive *Histoplasma* antigen in the urine (IMMY monoclonal antibody test); (ii)

confirmation by classical methods (microscopy, culture, or histopathology); or (iii) positive *Histoplasma* qualitative PCR (performed in accordance to [18]) in BAL samples, as well as bone marrow aspirates and tissue samples. Patients who are both antiretroviral treatment (ART) naïve and ART exposed will be recruited.

Exclusion criteria

Patients with previous diagnosis of histoplasmosis will not be considered for this study. We will also exclude pregnant or lactating woman, patients with renal failure (serum creatinine and urea >1.5x the upper limit of normality), abnormal aminotransferases (up to >3x the upper limit of normality), and patients with a previous serious reaction to a polyene antifungal drug. Patients who received more than one dose of a polyene antifungal drug in the last 48 hours will be excluded, as well as patients who refuse content to participate in the study. Patients diagnosed with histoplasmosis affecting the central nervous system will not be allowed to participate in the study. In addition, patients who are expected to die within 48 hours of enrolment will also be excluded, based on the judgment made by the attending physician. Patients diagnosed with tuberculosis will be excluded; as well those receiving drugs that cause significant drug-drug interaction (relative or absolute) with itraconazole.

Study settings

Six medical centers in Brazil, will recruit patients for this study: Santa Casa de Misericórdia de Porto Alegre, Hospital de Clínicas de Porto Alegre, Hospital Nossa Senhora da Conceição, Hospital de Doenças Tropicais, Hospital São Jose de Doenças Infecciosas, and Hospital Giselda Trigueiro.

Intervention

AIDS patients with DH will be randomized to one of three study arms: (i) single IV dose of L-AmB at 10 mg/kg; (ii) single IV dose of 10 mg/kg of L-AmB on day 1, followed by 5 mg/kg of L-AmB on day 3; and (iii) L-AmB at 3 mg/kg IV for 2 weeks. Induction therapy will be followed in all patients by oral therapy with itraconazole capsules at 400 mg/daily for a year.

Drug reconstitution and administration

L-AmB (AmBisome; Gilead Sciences, San Dimas, California, USA), a lyophilized liposomal preparation of amphotericin B, will be reconstituted according to the manufacturer's instruction and given intravenously at fixed doses, according to the randomization arm. L-AmB concentration should be of 0.2 mg/ml in 5% dextrose, and the drug should be infused over 2 hours, preferably in a central line. Patients will be monitored for the occurrence of infusion-related toxicity during and after drug infusion.

Primary endpoint

Clinical and mycological successes will be the primary outcomes for this study. A successful clinical response to induction therapy will be defined according to, as a maximum daily temperature <37.8°C for 72 hours; no increase in severity of clinical signs, symptoms, or laboratory abnormalities attributable to histoplasmosis; and the resolution of at least one of the signs or symptoms of histoplasmosis that qualified the patient for study enrollment [7].

Primary study outcome will be determined at day 14 of the study.

Secondary endpoints

Overall mortality and investigator-attributable mortality due to histoplasmosis will be determined at day 14 of study. Overall Survival (OS) will also be determined at 12, 24, and 48 weeks of study. OS will be considered as the time from randomization until death from any cause.

Secondary endpoint will also include need for an additional antifungal course with L-AmB, during 24 weeks of follow up.

Median percentage of change in renal and hepatic function at 14 days will be compared with baseline values.

Frequency of infusion-related toxicity with L-AmB will be recorded.

Clearance of blood cultures will not be used as a criterion for mycological response in this study, due to the limited sensitivity of blood culture in histoplasmosis [19]. Fungal clearance will also not be used as a criterion for mycological response. Differently from cryptococcosis, in which fungal burden is easily obtained and quantified in cerebrospinal fluid samples,

in histoplasmosis commonly a single positive sample is obtained along patient investigation (e.g., microscopy, culture, and/or histopathology).

Non-culture laboratory markers have proved to be useful adjuncts in monitoring the response to systemic histoplasmosis [20, 21]. In this study, we will evaluate the effect of a decrease of at least 50% in *Histoplasma* urinary antigen concentrations [19] along the first 2 weeks of therapy as a secondary endpoint, and the association of such reductions with clinical response to antifungal therapy.

Rescue therapy

The study protocol will allow patients to receive additional courses of L-AmB (at 3 mg/kg daily) in case the attendant physician suspect of absence of response with the induction regimen. This will apply for failing patients receiving a single 10 mg/kg of L-AmB; or 10 mg/kg on day 1, followed by 5 mg/kg on day 3. In these cases, L-AmB should be given up to a maximum period of 2 weeks. The estimated frequency of L-AmB as rescue therapy will be based on the frequency of failure with L-AmB at 3 mg/kg in patients with DH and AIDS (i.e., 12%) [7]. Considering that 66 patients will be evaluated in arms (i) and (ii) of the study (high-doses of L-AmB), in a pessimistic scenario we aim to provide 8 additional treatments with L-AmB.

Time for initiating ART

Physicians will be instructed to initiate ART as soon as possible in AIDS patients with DH, since immune reconstitution syndrome is uncommon in patients with DH [22]. In Brazil, initial ART consists of dolutegravir, lamivudine, and tenofovir. History of previous antifungal use and known resistance patters will also be considered in the decisions about ART.

Statistical analyses

Descriptive statistics will be used to summarize the data. Mean, standard deviation, median, interquartile range and minimum and maximum will be described for continuous variables. Categorical variables will be summarized by absolute and relative frequencies. Kaplan-Meier method will be used in order to describe OS. Despite the non-comparative status of the

trial, exploratory comparisons will be performed across subgroups. Continuous variables will be compared using two sample t-test, paired sample t-test, Mann-Whitney test, Wilcoxon signed-rank test, one-way ANOVA or Kruskal-Wallis test, as appropriate and necessary. Categorical variables will be compared with Fisher exact test or chi-square test, as appropriate. All p-values will be two-sided.

We expect that the three arms will have the same clinical response. For each arm, when the sample size is 29, a two-sided 90% confidence interval will extend 10% from the observed proportion for an expected proportion of response equal to 88% [as extracted from the Johnson trial]. Considering a dropout of 10%, the sample size per arm will be 33. The total sample size for the study will be 99 patients. Data analysis will be performed using SPSS software.

Ethical aspects

Conduction of this study is conditioned to ethical approval by local IRBs. Since this trial will not aim to modify any regulatory aspect related to L-AmB in Brazil, ANVISA (Brazilian Health Regulatory Agency) will not be consulted. The study will also be registered at clinicaltrials.gov.

Study activities

Clinical evaluations will take place at baseline, day 3, day 7, and day 14 (End of Study Visit). End of follow up will occur at week 48, and mortality will be determined at weeks 12, 24, and 48.

The study will recommend careful attention to hydration status, and close monitoring of renal function, hepatic function, and electrolytes. Unless clinically advisable, physicians are requested to give one liter of saline 0.9% solution intravenously before L-AmB infusion. Patients should also be on prophylaxis with daily trimethoprim-sulfamethoxazole.

Table 1 shows the procedures predicted for this study. Clinical and laboratory adverse events will be graded using the NIH DAIDS Toxicity Table [23]. Itraconazole therapeutic drug monitoring will be offered to sites at day 7 and day 14 of study. The attending physicians will use this information according to their personal judgment.

Table 1. Study activities.

	Baseline	Day 3	Day 7	Day 14	Week 12	Week 24	Week 48
Informed Consent	X						
History	X						
Physical Exam	X		X	X			
Maximum Temp	X	X	X	X			
Glasgow coma scale	X		X	X			
Chemistry (including magnesium) and blood counts	X		X	X			
HIV documentation	X						
CD4 count determination							
Pregnancy Test	X						
Chest X-ray	X			X			
Study Drug accountability	X						
Concomitant medications	X	X	X	X			
Adverse Events		X	X	X			
Survival determination				X	X	X	X
Molecular test for TB	X						
Urine for Histo antigen test	X		X	X			
Histoplasma PCR (as needed)	X						
Fungal culture (as needed)	X						
Microscopy (as needed)	X						
Histopathology (as needed)	X						
Itraconazole TDM			X	X			
Lysis centrifugation (if available)	X						
Study responde determination				X			
Need for additioal course of L-AmB				X	X		

Study Plan Schedule Strategy

Table 2 shows the predicted timeline for this study.

Table 2. Study plan.

	2018			2019											2020									
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Set	Out
Grant application	x																								
Expectation for grant approval			x																						
Project submission to IRBs					x	x																			
Registraton at clinicaltrials.gov							x																		
Expected IRBs approval								x																	
Arrival of Ambisome at sites							x	x																	
Importation of diagnostic kits							x	x																	
Patient enrollment									x	x	x	x	x	x	x	x	x	x	x	x	x				
Data analysis														x							x	x	x	x	x
Draft manuscript																									x
Article submission																									x
End of study																									x

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