



## Original article

## Determination of the therapeutic activity of caspofungin compared with the amphotericin B in an animal experimental model of histoplasmosis in hamster (*Mesocricetus auratus*)

Jorge Finquelievich\*, Maria Fernanda Landaburu, Victoria Pinoni, Cristina Adela Iovannitti

Microbiology Department, Mycology Center, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina

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## ABSTRACT

**Background:** Treatment with amphotericin B is highly effective in histoplasmosis. Caspofungin has shown good activity against *Candida* and *Aspergillus* spp. *In vitro* studies have demonstrated that *Histoplasma capsulatum* is inhibited by caspofungin.

**Objectives:** The purpose of this study was to evaluate the effectiveness of caspofungin in the treatment of histoplasmosis in an animal experimental model.

**Methods:** Three strains of *Histoplasma capsulatum* var. *capsulatum* were used. Treatment started one week post-inoculation and the animals were randomly assigned to six groups: amphotericin B 6 mg/Kg/d, caspofungin 2 mg/Kg/d, 4 mg/Kg/d, 8 mg/Kg/d and the other two groups received saline solution and dextrose solution. Blood samples for culture were obtained once a week, from day 7 to 35 post-inoculation. One week after the end of the treatment the animals were sacrificed and spleen cultures were performed. **Results:** Blood cultures were negative in all the hamsters which received amphotericin B (100%,  $P < 0.001$ ); those treated with caspofungin and the control animals presented 30 and 32% of positive cultures respectively ( $P = 0.59$ ). Spleen cultures were negative in the animals treated with amphotericin B, while the percentage of positive spleen cultures in the caspofungin groups varied from 25 to 100%, and in the control groups from 35 to 94.8% ( $P = 0.07$ ). The statistical analysis of the undiluted cultures showed the use of amphotericin B as the only independent predictor of negative culture ( $P < 0.001$ ).

**Conclusions:** The efficacy of amphotericin B is well known for the treatment of histoplasmosis, though we could not demonstrate that caspofungin is better than control.

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### Evaluación de la actividad de caspofungina comparada con anfotericina B en un modelo experimental de histoplasmosis en hámsters (*Mesocricetus auratus*)

## RESUMEN

**Antecedentes:** La anfotericina B es un fármaco efectivo para el tratamiento de la histoplasmosis. La caspofungina es activa contra especies de *Candida* y *Aspergillus*. Estudios *in vitro* han demostrado el efecto inhibitorio de este fármaco en *Histoplasma capsulatum*.

**Objetivo:** Evaluar la eficacia de la caspofungina comparada con la anfotericina B para el tratamiento de histoplasmosis en un modelo experimental en hámster.

**Métodos:** Se utilizaron tres cepas de *Histoplasma capsulatum* var. *capsulatum*. El tratamiento comenzó una semana tras la inoculación y los animales fueron distribuidos aleatoriamente en 6 grupos, según los fármacos utilizados: anfotericina B 6 mg/kg/día, caspofungina en dosis de 2, 4 y 8 mg/kg/día, solución fisiológica y dextrosa al 5%. Se tomaron muestras semanales para hemocultivos a partir del séptimo día postinfección y a los 7 días de finalizado el tratamiento los animales fueron sacrificados, realizándose cultivos del bazo.

## Palabras clave:

Tratamiento de la histoplasmosis

Caspofungina

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\* Corresponding author.

E-mail address: [jfinquel@yahoo.com.ar](mailto:jfinquel@yahoo.com.ar) (J. Finquelievich).

**Resultados:** Los resultados obtenidos de los hemocultivos realizados al finalizar el tratamiento fueron negativos en un 100% en el grupo tratado con anfotericina B, frente al 30% en el grupo tratado con caspofungina y el 32% en el grupo control ( $p=0,59$ ). Los cultivos de bazo fueron negativos en el grupo de anfotericina B, mientras que en el grupo de caspofungina los valores fluctuaron entre el 25 y el 100%, y en el grupo control entre el 35 y el 94,8% ( $p=0,07$ ). En el análisis estadístico del cultivo de la suspensión de bazo sin diluir el uso de anfotericina B fue el único predictor independiente de cultivos negativos ( $p=0,001$ ).

**Conclusiones:** La anfotericina B es un fármaco efectivo para el tratamiento de la histoplasmosis. La administración de caspofungina no demostró mayor eficacia en comparación con el grupo control.

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Histoplasmosis is an endemic systemic mycosis with high prevalence in Argentina. The development of progressive disseminated histoplasmosis depends on the host immunity, especially immunosuppressed patients who are unable to develop effective cell-mediated immunity. This includes patients with AIDS, transplant recipients, patients with hematological malignancies and those treated with corticosteroids.<sup>6</sup> In Argentina, it is the third fungal disease in AIDS patients. Amphotericin B and itraconazole are highly effective, with response rates of 88%.<sup>15</sup> However, amphotericin B is toxic and itraconazole has a variety of drug interactions and an erratic bioavailability. Therefore, it is important to explore new classes of drugs against histoplasmosis.

Caspofungin is a semi-synthetic derivate of pneumocandin B, a product of *Glarea lozoyensis*. This drug targets the cell wall, blocking the synthesis of  $\beta(1,3)$ -D-glucan by non-competitive inhibition of the enzyme  $\beta(1,3)$ -D-glucan synthase.<sup>8,13</sup> Caspofungin has shown good activity against *Candida* and *Aspergillus* spp. In vitro studies have demonstrated that *Histoplasma capsulatum* is inhibited by caspofungin. This drug offers an alternative to the azoles and avoids amphotericin B-associated toxicity.<sup>4,8,13</sup>

The purpose of this study was to evaluate the effectiveness of caspofungin in the treatment of histoplasmosis in an animal experimental model.

## Materials and methods

### Pathogen

Three strains of *H. capsulatum* var. *capsulatum* were used: they were named “hamster”, “non-AIDS” and “AIDS”, belonging to the Mycology Center Collection. The “hamster” strain is currently used as a standard strain for in vivo sensitivity with antifungal agents.<sup>3</sup> The other strains of recent isolation were derived from patients with disseminated histoplasmosis. We inoculated the strains in animals to establish the persistence of pathogenicity. The yeast obtained in this procedure was used for the inoculation.

### Animals and inoculation technique

A total of 360 hamsters (*Mesocricetus auratus*) of both sexes, weighting approximately 100 g, were used. They were anesthetized and inoculated intracardially with 0.1 ml of a suspension containing  $1 \times 10^7$  living cells/ml of the yeast phase, in isotonic saline solution. The viability of yeast phase cells was controlled by culturing the inocula in dextrose agar broth (Biokar Diagnostics, Francia) at 37 °C. The animals were maintained according to the Guide for the Care and Use of Laboratory Animals of the National Research Council.<sup>5</sup>

### Drug preparation

Amphotericin B (Sigma-Aldrich) was dissolved in dextrose solution at a final concentration of 2.5 mg/ml. Caspofungin (Merck Sharp & Dohme) was prepared in isotonic saline solution at a

concentration of 4 mg/ml, and then diluted to be used in three different doses: 2, 4 and 8 mg/kg of body weight. Caspofungin doses were based on toxicity and pharmacokinetic data from mice of previous reports on caspofungin.<sup>7</sup>

### Treatment schedule

The antifungal treatment started 7 days post-inoculation and was carried out for 3 weeks by intraperitoneal route once a day. The drugs used were amphotericin B 6 mg/kg/d at a volume of 0.25 ml, caspofungin 2, 4 and 8 mg/kg/d at a volume of 0.2 ml. Control animals received drug solvents at a volume of 0.2 ml (saline and dextrose solution).

Animals were randomly assigned to three groups of 120 hamsters each. The “hamster” strain was used in the first group, the “non-AIDS” strain in the second and the “AIDS” strain in the third. Each group was randomly assigned to six subgroups of 20 animals each, according to the different doses and drugs used.

### Treatment evaluation

The experimental mycosis outcome was controlled once a week by blood culture. Blood samples for culture were obtained at days 7-14-21-28 and 35 post-infection. A volume of 0.2 ml of blood was cultured in Sabouraud dextrose agar and incubated at 28 °C for 30 days, according to previous experiments.<sup>2</sup>

One week after the end of treatment (35 days), the animals were sacrificed and autopsy was performed. Response to therapy was determined by quantifying the viable yeasts. Homogeneous suspensions of the spleen in distilled water were prepared in sterile mortars, at a concentration of 100 mg/ml. For CFU determination, 0.1 ml of 1/10 and 1/100 dilutions of this original suspension were cultured in Petri dishes containing Sabouraud dextrose agar. The plates were incubated at 28 °C for four weeks and growth was controlled weekly.

Undiluted suspension of the spleen was performed in the same culture medium and temperature.

### Statistical analysis

Blood cultures were analyzed as contingency tables by means of the  $\chi^2$  test. ANOVA was performed for CFU comparison.<sup>9</sup> Since the variable for the amphotericin B group was always zero, this group was excluded. Additionally, a log transformation was used to reach the normality of the variable in each group assumption. For statistical analysis of the undiluted cultures, a stepwise backwards logistic regression was implemented with a break-point of 0.05 as the probability to exist. The presence or absence of microorganisms in massive spleen cultures was used as the predicted variable. Dummies were created, indicating the use of each of the six treatment variants (amphotericin B, caspofungin at three different concentrations, isotonic saline solution and dextrose solution), and the three inoculated strains

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