Journal de Mycologie Médicale (2017) xxx, xxx—xxx

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

ORIGINAL ARTICLE/ARTICLE ORIGINAL

Modulatory effect of voriconazole on the production of proinflammatory cytokines in experimental cryptococcosis in mice with severe combined immunodeficiency

E. Gonçalves Silva [a,*], S. Marilia de Souza Silva [b], C. Rodrigues Paula [a], L. da Silva Ruiz [c], A. Latercia Tranches Dias [d]

[a] University of São Paulo, Faculdade de Odontologia — FOUSP, avenida Professor Lineu-Prestes, 2227, Cidade Universitária, CEP: 05508-000, São Paulo, Brazil
[b] Universidade Federal do ABC — UFABC, avenida dos Estados, 5001 Bairro Santa Terezinha, Santo André, 09210-580 São Paulo, Brazil
[c] Instituto Adolfo Lutz, Bauru, rua Rubens Arruda, quadra 6, Altos da Cidade, 17015-110 Bauru, Brazil
[d] Universidade Federal de Alfenas, Alfenas, rua Gabriel Monteiro da Silva, 700 Alfenas, 37130-001 Minas Gerais, Brazil

Received 16 September 2017; accepted 29 November 2017

KEYWORDS
Cryptococcosis;
Cytokines;
SCID;
Voriconazole;
Amphotericin B;
Cryptococcus neoformans

Summary Cryptococcosis is a subacute or chronic disease. For many years, amphotericin B has been used in severe fungal infections. Voriconazole is a triazole with high bioavailability, a large distribution volume, and excellent penetration of the central nervous system (CNS). The objective of this study was to evaluate the production of pro-inflammatory cytokines in the lungs during an experimental infection caused by *C. neoformans* in murine model (SCID) that was treated with amphotericin B and voriconazole. After intravenous inoculation with 3.0×10^5 viable yeast cells, the animals were treated with amphotericin B and voriconazole. The daily treatments began 24 hours after inoculation and lasted 15 days. We evaluated the survival curve and we measured the levels of TNF-α, IL-6 and IL-10. For all treatments, there was a significant increase in survival compared to the untreated group of animals and the group treated with voriconazole (maximum concentration). The levels of pro-inflammatory cytokines were significantly lower in the groups treated with voriconazole (maximum concentration) and amphotericin B (minimum concentration). Under the conditions studied, we can suggest by that the

* Corresponding author.
E-mail address: eriques@usp.br (E. Gonçalves Silva).

https://doi.org/10.1016/j.mycmed.2017.11.008

Please cite this article in press as: Gonçalves E, et al. Modulatory effect of voriconazole on the production of proinflammatory cytokines in experimental cryptococcosis in mice with severe combined immunodeficiency. Journal De Mycologie Médicale (2017), https://doi.org/10.1016/j.mycmed.2017.11.008

production of pro-inflammatory cytokines mediated by amphotericin B and voriconazole is dependent on the concentration administered.

Introduction

Cryptococcus neoformans variety *neoformans* is a pathogenic opportunistic yeast that is commonly found in the excreta of pigeons and other birds [1]. The yeast is responsible for subacute or chronic systemic mycoses termed ''cryptococcosis'' (Casadevall A, Perfect JR. *Cryptococcus neoformans*. Washington, DC: ASM Press, 1998 [2]). The diseases are caused by inhaling infective particles of yeast, which remain in the lungs and cause pulmonary cryptococcosis (Casadevall A, Perfect JR. *Cryptococcus neoformans*. Washington, DC: ASM Press, 1998 [2]). *C. neoformans* spreads to other organs, mainly the central nervous system (CNS), through the bloodstream (Casadevall A, Perfect JR. *Cryptococcus neoformans*. Washington, DC: ASM Press, 1998 [2]). Mice with severe combined immunodeficiency (SCID) are more susceptible to systemic experimental cryptococcosis and can be a good model to study the immunologic response and therapeutics [3]. The higher susceptibility of this model is due to lack of B cells and T cells that contribute to protect this model against the severe disease [4]. Tumor necrosis factor (TNF-α) is an important marker in cryptococcosis in both humans and the murine model [5]. TNF-α is a pro-inflammatory cytokine with various biological functions, including increasing the modulating the expression of other cytokines, such as IL-1, IL-1 (beta) and IL-6, that are secreted by macrophages, neutrophils and T cells [6]. The increased levels of IL-6 occur within three days of treatment with amphotericin B in cryptococcosis [7].

Amphotericin B, fluconazole and 5-fluorocytosine are most commonly used drugs to treat cryptococcosis [8]. For more than 30 years, amphotericin B has been used in severe fungal infections, but its use has been limited because of the various adverse effects, most importantly its nephrotoxicity [9,10]. Fluconazole is used to treat cerebral cryptococcosis, [11] but long-term indiscriminate use can result in resistance to this drug [12]. Voriconazole is a triazole with high bioavailability, a large distribution volume, excellent penetration of the CNS, [13] excellent in vitro activity against *C. neoformans* [14] and good concentrations in the serum [11].

The objective of this study is to evaluate the pro-inflammatory cytokines production in the lungs in an experimental infection caused by *C. neoformans* in a (SCID) murine model treated with amphotericin B and voriconazole.

Materials and methods

Cryptococcus neoformans strain

The studies were performed using the *C. neoformans* strain ATCC 90112 (serotype A). This strain was maintained in tubes containing Sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA) and glycerol at −20 °C in the Laboratory of Pathogenic Yeasts of the Department of Microbiology, Institute of Biomedical Sciences of São Paulo University, São Paulo, Brazil [15].

Animal model and experimental cryptococcosis

Forty-five BALB/c-SCID (severe combined immunodeficiency) mice with a mean weight of 20 g were obtained from the Animal Center, which was responsible for breeding isogenic animals at the Institute for Energy and Nuclear Research, São Paulo, Brazil. These mice were housed in microisolator cages, provided with sterile food and water and randomly distributed into IN LINE 4 IN Modèle animal et cryptococcose expérimentale mettent six groupes. IN LINE 4 IN Animal model and experimental cryptococcosis put six groups. X groups. The strain (ATCC 90112) was cultivated in YPD medium (1% yeast extract (Difco), 1% Bacto Peptone (Difco) and 2% dextrose (Sigma-Aldrich, Milwaukee, WI, USA) for 18 h at 30 °C; the cells were collected by centrifugation, washed twice in a phosphate buffer solution (PBS) and resuspended at the inoculation concentration. Five groups (with 5 animals each), were inoculated intravenously with 100 μL of the suspension containing 3.0×10^5 viable yeast cells. Among these groups, four groups were treated, and one group (with 10 animals), was not treated, and served as the positive control. One group (with 10 animals) was inoculated with PBS and served as the negative control. Animal handling and treatments followed the Ethical Principals of the Brazilian College of Animal Experimentation (COBEA) [15].

Treatments

The daily treatments began 1 day after the initial inoculation and lasted 15 days. The animals were inoculated intraperitoneally with 0.1 mL of amphotericin B (0.75 or 1.5 mg/kg/day) (Fungizone, Bristol-Meyers, Squibb S.p.A., Sermoneta, Italy), [16] or 0.1 mL of voriconazole (20.0 or 40.0 mg/kg/day) (Vfend® IV) (Pfizer Inc, New York, NY, USA) [17]. At the end of study period (50 days), all animals that survived were euthanized in a CO_2 chamber. The dead and surviving mice were evaluated by survival curves and cytokine levels (TNF-α, IL-6 and IL-10) in lung homogenates.

Removal and homogenization of lungs

The lung were aseptically removed, weighed and homogenized in a solution containing 1.0 mL of phosphate buffer solution (PBS) supplemented with 0.05% Tween 20, 1% protease inhibitor (Sigma-Aldrich) and 1% phenylmethylsulfonyl fluoride (PMSF-1 mM) (Sigma-Aldrich). They were centrifuged for 5 minutes at 14,000 rpm and organ homogenates that were not used immediately were stored at −80 °C for later use.

Please cite this article in press as: Gonçalves E, et al. Modulatory effect of voriconazole on the production of proinflammatory cytokines in experimental cryptococcosis in mice with severe combined immunodeficiency. Journal De Mycologie Médicale (2017), https://doi.org/10.1016/j.mycmed.2017.11.008