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ORIGINAL ARTICLE

Intravenous itraconazole against experimental neutropenic *Candida parapsilosis* infection: efficacy after suppression of bacterial translocation

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Abstract A variety of studies indicate that itraconazole possesses greater intrinsic activity compared to the other azole derivatives against *Candida parapsilosis*. Efficacy has never been tested in an experimental setting. To this end, *C. parapsilosis* was used for challenge of 117 rats rendered neutropenic after a course of cyclophosphamide. Rats were assigned to receive intravenous treatment with saline (group A); itraconazole q12h (group B); fluconazole q12h (group C); single dose of ceftriaxone and saline (group D); single dose of ceftriaxone and itraconazole q12h (group E); and single dose of ceftriaxone and fluconazole q12h (group F). Survival was recorded, and yeast outgrowth of liver, spleen, lung, and kidney was measured after sacrifice at serial time intervals. Growth of the test isolate in tissues was significantly lower in group B than in groups A and C after 72 h. However, outgrowth of enterobacteria was found in tissues of groups A, B, and C, implying a phenomenon of bacterial translocation from the gut. When this phenomenon was suppressed with single doses of ceftriaxone, a striking survival benefit of itraconazole-treated animals was found ($p = 0.022$, group E vs. group F). The present results suggest than in deep infections by *C. parapsilosis* intravenously administered itraconazole may eradicate the offending agent and provide survival benefit when chemotherapy-induced bacterial translocation from the gut is suppressed. Further clinical evidence is required to support these findings.

Keywords Itraconazole · *Candida parapsilosis* · Experimental infection · Fluconazole

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Introduction

Fungal bloodstream infections are a common cause of hospital-acquired infections. They mostly affect neutropenic and immunocompromised hosts but they can also affect nonneutropenic patients who are hospitalized in an intensive care unit (ICU). Among patients in the ICU, *Candida* spp. are the fourth most common cause of sepsis [1]. Traditionally, *Candida albicans* was considered the most common cause of candidemia. However, the broad use of fluconazole for prophylaxis and the inherent resistance of many *Candida* species to the azole antifungals have led to a switch of the epidemiology. Many recent publications from Brazil, Germany, Greece, Italy, Spain, and the United States report emergence of non-*albicans Candida* spp. in more than 50 % of cases of bloodstream infections in both neutropenic and nonneutropenic hosts [2–6].

Candida parapsilosis has emerged as the cause of 8–50 % of all cases of candidemia [4–6]. Using molecular genotyping, three subspecies have been typed, namely, *C. parapsilosis* sensu stricto, *C. metapsilosis*, and *C. orthopsilosis*. *C. parapsilosis* sensu stricto is the most

common pathogen among species of the *C. parapsilosis* complex. In general, azoles are the treatment of choice for invasive fungal infections by *C. parapsilosis* whereas echinocandins are not recommended as a first-line drug [7]. Recent reports on the susceptibilities of all azole antifungals against *C. parapsilosis* indicate that minimum inhibitory concentrations (MICs) of itraconazole are lower than those of fluconazole and of posaconazole, implying that itraconazole possess a greater intrinsic activity against that species [2, 8, 9]. Despite in vitro data, data about the management of infections by *C. parapsilosis* with itraconazole are lacking. This gap may be explained in part by the availability of parenteral formulations of itraconazole in a few countries that are hampered by poor drug water solubility whereas hospitalized patients with severe infections cannot be treated with an agent that is available only in an oral formulation.

The present study investigated the efficacy of an intravenous formulation of itraconazole in an experimental model of infection by *C. parapsilosis* in rats. Animals were rendered neutropenic to reproduce the clinical situation of neutropenic patients post chemotherapy who are prone to fungal infections. The efficacy of itraconazole was studied in light of the phenomenon of translocation of the bacterial flora from the gut that may take place when the integrity of the intestinal mucosa is disrupted after chemotherapy.

Animals and methods

Test isolate

One blood isolate of *Candida parapsilosis* from a patient with severe sepsis was used for the study. The isolate was typed by the API/ID32C system (BioMérieux, Marcy l'Etoile, France). Minimum inhibitory concentrations (MICs) of itraconazole, fluconazole, voriconazole, posaconazole, caspofungin, anidulafungin, micafungin, and amphotericin B measured by the microdilution technique (TREK Diagnostic System, East Grinstead, UK) were 0.06, 1, 0.015, 0.03, 0.5, 2, 2, and 0.25 μg/ml respectively.

Single colonies were suspended in RPMI1640 broth (Biochrom, Berlin, Germany) supplemented with dextrose 0.2 % and incubated for 12 h at 37 °C in a shaking water bath. The inoculum was adjusted to 5×10^7 cfu/ml by using 0.5 of the McFarland climax.

Animals

A total of 117 male Wistar rats (mean ± SD weight, 283.2 ± 54.0 g) were studied. The study was conducted in the Laboratory for Experimental Medicine of the ATTIKON University Hospital (Athens, Greece). The study protocol received a permit from the Veterinary Directorate of the Prefecture of Athens according to the Greek legislation in conformance to the 160/1991 Council Directive of the EU (license 2549/EL 25 BIO 014). Animals were housed in single metal cages with laminar air flow and had access to sterile water and standard balanced rabbit chow ad libitum. Room temperature ranged between 18 ° and 22 °C, relative humidity between 55 % and 65 %, and the light–dark cycle was 6 am–6 pm.

Study design

Animals were rendered neutropenic, as already described by our study group [10]. Briefly, two doses of cyclophosphamide (Aventis, Paris, France) were administered intraperitoneally: the first dose of 150 mg/kg was given 96 h before the challenge with the test isolate and the second dose of 100 mg/kg was given 48 h before challenge with the test isolate. Achievement of neutropenia, i.e., less than 1,000 neutrophils/mm^3, was confirmed in four rats by blood sampling 48 h after the second dose of cyclophosphamide and analysis through an automated counter (Coulter, Miami, FL, USA).

After induction of neutropenia, rats were challenged by the intravenous injection of 0.5 ml of the prepared inoculum of the test isolate in the tail vein under slight ether anesthesia. On each day, experiments were performed in two animals of each of the study groups mentioned next. Animals were randomly assigned into six groups of treatment, as follows:

- Group A ($n = 30$): controls, rats administered intravenously 0.5 ml water for injection q12h by the tail vein starting 1 h after challenge with the test isolate.
- Group B ($n = 30$): rats administered intravenously 10 mg/kg itraconazole (Micronazol; Hospital Line, Greece) q12h at a final volume of 0.5 ml by the tail vein starting 1 h after challenge with the test isolate. The dose of itraconazole was selected in analogy to former studies in rats and leads to serum levels comparable to the conventional doses for humans [11, 12]. Itraconazole was provided in vials of 10 ml containing 250 mg trichloric salt per vial, which was diluted with 0.9 % NaCl according to the instructions of the manufacturer.
- Group C ($n = 18$): rats administered intravenously 10 mg/kg fluconazole (Fungustatin; Pfizer, Greece) q12h at a final volume of 0.5 ml by the tail vein starting 1 h after challenge with the test isolate. The dose of fluconazole was selected in analogy to former studies in rats and leads to serum levels comparable to the conventional doses for humans [13].
- Group D ($n = 12$): rats administered intramuscularly a single dose of 100 mg/kg ceftriaxone (Roche, Athens,