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Lack of Ritonavir Antifungal Effect In Vitro

Oropharyngeal candidiasis (OPC) is the most common opportunistic infection in HIV-infected patients and is considered a clinical marker of disease progression (3). A reduction in the incidence of OPC and some cases of resolution of refractory mucosal candidiasis following introduction of HIV protease inhibitor (HIV-PI) therapy have recently been reported (6, 10, 11). To switch from saprophytic to pathogenic behavior, *Candida* has to increase its adhesive properties to attach to host components and its production of lytic enzymes to penetrate the tissues (9). The chief hydrolytic enzyme involved in *Candida* virulence is secretory aspartyl proteinase (5). An increased concentration of *Candida* aspartyl proteinase has been detected in isolates from oral cavities of HIV-infected patients (2). It has been suggested that HIV-PIs could have an additional effect on fungal proteases (6), since the target of HIV-PIs is also an aspartyl proteinase. We studied the sensitivity of *Candida albicans* to ritonavir, applying the National Committee for Clinical Laboratory Standards broth dilution test (7), in order to evaluate in vitro the antifungal effects of this antiretroviral agent.

Conventional petri plates with Sabouraud dextrose agar culture medium (batch 3209) were inoculated with a standard strain of *C. albicans* (strain ATCC 64550). Pure ritonavir (ABT-C38; Abbott, Chicago, Ill.) was diluted in ethanol to concentrations of 0.1, 0.2, 0.5, and 1 mg/liter. Sterile blank disks (Difco, Detroit, Mich.) containing the different dilutions of ritonavir were applied to the inoculated plates and then incubated at 35°C for 48 h. Each inoculated plate was examined for yeast growth inhibition at 24 and 48 h. For each ritonavir dilution the procedure was performed three separate times and the results were compared for reproducibility. Inhibitory zones were not observed in any plate after either 24 or 48 h.

It has been recently shown, in an experimental mucosal infection, that indinavir and ritonavir had an anti-*Candida* effect which appeared to be mediated by inhibition of *Candida* aspartyl proteinase activity (1). In our study, however, ritonavir did not show any direct fungicidal effect, not even when it was used at concentrations 10-fold those of the HIV-1 90% inhibitory dose (0.1 mg/liter). The results of the in vitro studies should be interpreted with caution, since the predictive value of "susceptibility" in laboratory tests depends mainly on the nature of the host, the specific site of infection, and the pharmacokinetics of the agent in a particular host (8). HIV-1 envelope proteins (gp160 and gp41) enhance the virulence of *C. albicans*, by increasing the secretion and activity of *Candida* aspartyl proteinase, and block the activity of phagocytic cells (4). We believe that the reduction of the frequency of HIV-related OPC following HIV-PI therapy is caused by a significant reduction in HIV-1 replication and by a recovery of the immune system favoring a predominantly Th_1-type response pattern and an increase in the number and activity of phagocytic cells.

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P. Diz
School of Medicine and Dentistry
Santiago de Compostela University
Santiago de Compostela
Spain

A. Ocampo
I. Iglesias
I. Otero
Complejo Hospitalario Xeral-Cíes
Vigo
Spain