TITLE: THE ANTIPROLIFERATIVE PEPTIDE CTN[15-34], A CATHELICIDIN-DERIVATE, IS EFFECTIVE AGAINST FLUCONAZOLE-RESISTANT YEASTS OF *CRYPTOCOCCUS NEOFORMANS*

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ABSTRACT: Crotalicidin [Ctn], an cathelicidin-related antimicrobial peptide from the venom gland of South American rattlesnake, as well as its C-terminal Ctn[15-34] fragment, have shown important activity against microorganisms including yeast species (*Candida* ssp. and *Crypcotoccus laurentii*). In this study, we investigated their activity against strains of fluconazole-resistant *C. neoformans*. The minimum inhibitory concentration (MIC) was established by the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute-CLSI (formerly NCCLS; M27-A2) and BacTiter-Glo luminescent cell viability tests were used to determine the inhibitory potential of the peptides and the susceptibility of pathogenic yeast strains to antifungal agents. As assay control, Fluconazole (FLC) was used. The peptides were solubilized in deionized water at a concentration of 80 µM, diluted in RPMI 1640 medium (Sigma) to adjust the test concentration from 0.02 to 40 µM. The microplates were incubated at 35

°C and read visually after 48 h. After incubation, 50 µl of BacTiter-Glo microbial cell viability assay reagent (Promega, Madison, WI, USA) was added to each well, and the plates were again incubated for 15 minutes at room temperature with gentle shaking. Luminescence was then measured in a BioTek Synergy HT reader (BioTek, Winooski, VT, USA). Relative cell viability was calculated with cells treated with RPMI as controls (~100% viability). The assays were carried out in triplicate. All peptides were active against *Cryptococcus* spp. strains, including those resistant to antifungals, with MICs ranging from 1.25 to 10 µM. Treatments of *C. neoformans* with 5 µM Ctn [15-34] resulted in only 7.5% viability. FLC treated showed some reduction after 50 µM concentration. With 200 µM the viability was still more than 40%. Overall, the crotalicidins and short fragments, in particular Ctn[15-34], are potential antifungal peptides, since they are active even against clinical isolates endowed with resistance mechanisms.

Keywords: antimicrobial peptide (AMP); *Cryptococcus neoformans*; crotalicidin; microbial cell viability assays; resistant yeasts.

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