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Antimicrobial Peptides Are Lytic To Acanthamoeba Castellanii

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Purpose: Acanthamoeba species are an important cause of keratitis, mainly in contact lens wearers. Because of its poor response to conventional antimicrobial agents at concentrations tolerated by the eye the outcome is generally severe visual impairment. We evaluated the in vitro efficacy of two classes of antimicrobial peptides against Acanthamoeba castellani trophozoites compared to rabbit corneal epithelial (SIRC) cells.

Methods: We used Gomesin, a b-hairpin peptide, and peptides derived from the N-terminus of trialysin (P5), which form amphipathic a-helix structures. Amoebicidal activity was investigated after incubation of A.castellanii trophozoites with different concentrations of the peptides and monitored by trypan blue test and flow cytometry for propidium iodide fluorescence. Growth inhibition was assessed during 6 days of incubation in 96-well plates. SIRC cells (ATCC CCL60) viability after exposure to peptides was done by MTT colorimetric assay. Degradation of peptides exposed to trophozoites supernatants was analyzed by liquid chromatography-mass spectrometry. To determine whether proteases inhibition enhanced the lytic effects of peptides, trophozoites were treated with phenylmethylsulphonyl fluoride and incubated in the absence or presence of peptides.

Results: Gomesin was more effective in promoting amoeba (LC50 = 15 μM) than SIRC cells permeabilization (LC50 = 25 μM), resisting proteolytic degradation. It was less effective in preventing growth because its action decreased in amoeba growth medium. P5 eptide promoted amoeba permeabilization at higher concentrations (LC50 = 36 μM) and was very sensitive to proteases secreted by amoeba. Nevertheless, peptide P5 prevented amoeba growth at concentrations as low as 5 μM. Addition of PMSF increased P5 lytic efficiency.

Conclusions: We concluded that although β-hairpin peptides are effective to kill amoeba at safe concentrations, their effect depends on the culture medium, which increases parasite resistance to lysis. In contrast, amphiphathic a-helix

peptides are effective in preventing growth but their action would depend on the susceptibility to amoeba proteases.