2007 Research Days Abstract Form – Department of Ophthalmology – UNIFESP/EPM

2. SCIENTIFIC SECTION PREFEREN CE (REQUIRED): Review the Scientific section Descriptions. Select and enter the two -letter Code for the one (1) Section best sullied to review your abstract
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3. PRESENTATION PREFERENCE (REQUIRED) Check one (1)
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Signature of First

Scientific Section Descriptions

(OR) ORBIT
(PL) OCULAR PLASTIC SURGERY
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(RX) REFRACTION-CONTACT LENSES
(NO) NEURO-OPHTHALMOLOGY
(TU) TUMORS AND PATHOLOGY
(ST) STRABISMUS
(UV) UVEITIS
(LS) LACRIMAL SYSTEM
(LV) LOW VISION
(CO) CORNEA AND EXTERNA L DISEASE
(GL) GLAUCOMA
(RS) REFRACTIVE SURGERY
(CA) CATARACT
(US) OCULAR ULTRASOUND
(TR) TRAUMA
(LA) LABORATORY
(BE) OCULAR BIOENGINEERING
(EP) EPIDEMIOLOGY

Deadline: 29/10/2007

FORMAT:
Abstract should contain:
Title, Name of Authors, Name of other authors (maximum 6), Purpose, Methods, Results, Conclusions.
Example: ARVO (1.10 x 1.70)
Abstract Book

1. FIRST (PRESENTING) AUTHOR (REQUIRED)
Must be author listed first in body of abstract

( ) R1 ( ) R2 ( ) R3
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Last Name	First Name	Middle
SACRAMENTO	ROGERIO	SILVA DO

Service (sector)	Nº CEP
CORNEA	1668/06

Title: Antimicrobial Peptides Are Lytic To *Acanthamoeba Castellanii*

Sacramento RS; Freitas D; Martins RM; Foronda A; Alvarenga L; Dobroff AS; da Cunha JPC; Rodrigues EG; Mortara, R;Miranda A; Schenkman S

Purpose: *Acanthamoeba* species are an important cause of keratitis, mainly in contact lens wearers. Because of its po or response to conventional antimicrobial agents at concentrations tolerated by the eye the outcome is generally severe visual impairment. W e 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 β-hairpin peptide, and peptides derived from the N-terminus of trialysin (P5), which form amphipathic α-helix structures. Amoebicidal activity was investigated after inc ubation 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 c ells (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 ofpeptides.

Results: Gomesin was more effective in promoting amoeba (LC_{50} = 15 ìM) than SIRC cells permeabilization (LC_{50} = 25 ìM), 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 (LC_{50} = 36 ìM) and was very sensitive to proteases secreted by amoeba. Nevertheless, peptide P5 prevented amoeba growth at concentrations as low as 5 ìM. Addition of PMSF increased P5 lytic efficiency.

Conclusions: We concluded that although â -hairpin peptides are effective to kill amoeba at sa fe concentrations, their effect depends on the culture medium, which increases parasite resistance to lysis. In contrast, amphiphathic α-helix peptides are effective in preventing growth but their action would depend on the susceptibility to amoeba proteases.