Characterization of dual effects induced by antimicrobial peptides: regulated cell death or membrane disruption

Paredes-Gamero, E. J.¹,²*, Martins, M.N.C.²*, Cappabianco, F.A.M.³; Ide J.S.³, Miranda, A²#.

¹Departamento de Bioquímica and ²Departamento de Biofísica, UNIFESP, São Paulo, SP, Brazil. ³Departamento de Ciência e Tecnologia, UNIFESP, São José dos Campos, SP, Brazil.

Recently, studies have focused on the antitumoral activities of the antimicrobial peptides (AMPs). However, the mechanisms by which AMPs regulate cell death in mammals are not well understood. In this study, we investigated the cell death-inducing activities of four β-hairpin AMPs (gomesin, protegrin, tachyplesin, polyphemusin II) along with their linear analogues, and magainin II, a linear AMP, in the human erythroleukemia K562 cell line. Gomesin and protegrin displayed cytotoxic properties that their linear counterparts did not. Interestingly, tachyplesin and polyphemusin and also their linear analogues induced cell death. We were able to distinguish two ways in which these AMPs induced cell death depending on their concentration range utilized. Lower concentrations of AMPs induced controlled cell death mechanisms such as apoptosis, secondary necrosis and necrosis/necroptosis. Each AMP tested promoted controlled cell death by different intracellular mechanisms. Gomesin, tachyplesin and Linear-tachyplesin promoted apoptosis that was characterized by annexin labeling, sensitivity to Z-VAD, and caspase-3 activation. Gomesin and protegrin induced cell death were also dependent on intracellular Ca²⁺ mechanisms. The mediated cell death induced by gomesin, tachyplesin and Linear-polyphemusin II was inhibited by necrostatin-1. Conversely, treatment with higher concentrations of AMPs, above the EC₅₀, primarily resulted in cell
membrane disruption, but with different patterns of action for each AMP tested. Thus, at low concentrations of AMPs the induction of the controlled cell death prevails, but at higher concentrations of it the direct disruption of the cell membranes is the most favorable mechanism.

**Keywords:** antimicrobial peptide, cell death, membrane permeabilization, intracellular mechanism.