Spectroscopic studies of membrane perturbation induced by the antimicrobial peptidomimetic NCK-10

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For over 70 years, antibiotics revolutionized medicine, but their abuse in human therapy, husbandry and agriculture is causing a drastic increase in bacterial drug resistance. Natural antimicrobial peptides (AMPs) represent a possible solution to this issue, because they do not act by associating to a specific protein target, like traditional antibiotics, but by perturbing the permeability of bacterial membranes. However, these peptides have some limitations for clinical use, due to their susceptibility to proteolytic degradation.

In this work, we characterized a peptidomimetic molecule, termed NCK-10, which maintains the characteristics of natural AMPs, with improved pharmacological properties. Spectroscopic studies, using liposomes as model membranes, showed that the membrane perturbation mechanism of NCK-10 is similar to that of antimicrobial peptides – the compound forms pores in membranes by inserting under the polar heads of phospholipids and thus perturbing the surface tension of the outer leaflet. Furthermore, this molecule perturbs the fluidity of the membrane and causes vesicle aggregation. Both these effects probably contribute to its bactericidal activity. Literature results regarding the selective activity of NCK-10 against bacteria in *in vivo* and *in vitro* studies are contradictory. To clarify this aspect, we studied the interaction of NCK-10 with membranes of different lipid compositions and the pore-forming activity of the peptidomimetic resulted to be independent on membrane charge, intrinsic curvature or sterol content. Through *in vitro* studies, we found that the bactericidal activity and toxicity towards eukaryotic cells of NCK-10 are both dependent on the density of cells. Since very different cell concentrations are used in bactericidal and haemolytic activity assays, this finding could explain the apparent selectivity observed in previous *in vitro* studies. Therefore, our data indicate that the currently used protocols for the determination of the cell selectivity of AMPs and peptidomimetics should be revisited.