Synergistic effect of membrane-active peptides SP-A and SP-BN on multidrug-resistant Klebsiella pneumoniae

P02-06

V. Fraile Ágreda[I,II], O. Cañadas Benito[I,II], C. Casals Carro[I,II]

[I]Universidad Complutense de Madrid, Madrid, Spain, [II]Centro de Investigaciones Biomédicas en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain

The emergence of multi-resistant strains of the respiratory pathogen *Klebsiella pneumoniae* underlines the need to implement new non-antibiotic therapies, including the characterization of natural antibacterial proteins as a paradigm for endogenous defense pathways. We have previously shown that lung surfactant protein (SP-A), acting synergistically with the lung anti-microbial peptide SP-B^N, enhances capsulated *K. pneumoniae* K2 clearance *in vivo*, in part by contributing to bacterial killing. However, the factors that govern SP-A/SP-B^N anti-microbial activity are still unclear. The aim of this work was to study the mechanism by which SP-A and SP-B^N exert a synergistic microbicidal activity against capsulated *K. pneumoniae*, which is otherwise resistant to either protein alone. Our results indicate that the SP-A/SP-B^N complex, but not the individual proteins, alters the bacterial ultrastructure, forming pores in the membrane that favor the translocation of both proteins to the periplasmic space, where they interact with the inner membrane, inducing its depolarization and fission into small vesicles. *In vitro* studies with model membranes, which mimicked the internal and external bacterial membranes, showed that both SP-A and SP-B^N bound to lipopolysaccharide molecules present in the outer membrane, inducing lipid phase separation and disrupting membrane packing. This effect was stronger for the SP-A/SP-B^N complex, which rendered both the outer and inner bacterial membranes leaky as determined by permeabilization studies. Finally, the SP-A/SP-B^N-induced fission of the inner membrane may be related to the promotion of positive curvature observed by differential scanning calorimetry. Taken together, our results indicate that the antimicrobial activity of the SP-A/SP-B^N complex is related to its capability to alter the integrity of outer and inner bacterial membranes.