IJAE
Vol. 120, n. 1 (Supplement): 83, 2015
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Effect of Chitlac-nAg on Streptococcus mitis internalization into human gingival fibroblasts

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The surfaces of the oral cavity are always exposed to a broad variety of microorganisms able to form biofilms (Filoche et al, 2010) characterized by microbial communities that are organized as a network of cell-to-cell interactions. Streptococci are the predominant bacterial population of the oral environment and *S.mitis* in particular is the first colonizer of the oral biofilm (Di Giulio et al, 2013). Silver-based medical products have been proven to be effective in retarding and preventing bacterial growth. In order to prevent silver nanoparticles aggregation, a lactose-modified chitosan has been set up and resulted effective in stabilizing colloidal solution of nanoparticles (Chitlac-nAg) (Travan et al, 2009). Since many bacteria are able to internalize into eukaryotic cells, in our study we have investigated both the intracellular signaling governing *S. mitis* internalization into HGFs and the biological effect of Chitlac-nAg on eukaryotic and prokaryotic cells in a co-culture model system.

The internalization of *S. mitis* into HGFs is due to F-actin cytoskeleton reorganization and reduced expression within the cell. Immunofluorescence shows actin polymerization at invasion sites along with vinculin increased expression and spot organization. Vinculin is an adaptor protein that regulates the adhesion of integrin receptors to actin cytoskeleton. In presence of *S. mitis* an increment of integrin β1 and FAK expression, responsible for the entrance of the microorganism in HGFs is consistent, as revealed by electron microscopy analysis. This adhesion and uptake proteins profile is the same in the presence of saliva as well as bacteria uptake. When Chitlac-nAg is administred to cell culture the expression of all four proteins decreases and Ag nanoparticles are recognized within the cells. Further, in presence of Ag nanoparticles the low amount of FAK is almost localized at nuclear level. In presence of Ag and *S.mitis*, the expression of all four proteins is increased, with respect to control, and F-actin cytoskeleton rearranged, while a raised number of bacteria is shown. This effect is mitigated by the presence of saliva in cell culture, which probably prevents bacteria entry into the cell. These results let us hypothesize that Chitlac-nAg, developing its bactericidal action could represent a good component of tooth paste and mouthwash.

References

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Keywords

Chitlac-nAg; human gingival fibroblasts; Streptococcus mitis; internalization; focal adhesion kinases; vinculin; β1 Integrin; f-actin.