TITLE: ROLES OF PepO AND CppA IN THE *Streptococcus sanguinis* ABILITY TO INVADE HUMAN CORONATY ARTERY ENDOTHELIAL CELLS.

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ABSTRACT:

Streptococcus sanguinis is an abundant commensal species of the oral cavity commonly involved in opportunistic cardiovascular infections, through unknown mechanisms. There is evidence that *S. sanguinis* express proteases for evasion to complement system, which further participate in bacterial invasion into endothelial cells, two important functions for cardiovascular infections. The aim of this study is to investigate the roles two proteases of complement proteins, PepO and CppA in *S. sanguinis* ability to invade primary human coronary artery endothelial cells (HCAEC). To this end, isogenic mutants of *pepO* and *cppA* were obtained in the invasive *S. sanguinis* strain SK36, by doble cross-over recombination with null alleles. These mutants were then compared with the parent strain regarding the frequency of invasion of primary human coronary artery endothelial cells (HCAEC), using antibiotic protection assays. Briefly, HCAEC seeded in 24-well flat-bottomed tissue culture plates were exposed to 1×10^7 CFU of *S. sanguinis* strains (MOI 1:100) in antibiotic-free medium during 2h (37ºC, 5% CO_2). Afterwards, cells were washed and incubated in medium containing penicillin G and gentamycin (1 hour, 37ºC, 5% CO_2) for killing of extracellular bacteria. HCAEC cells were then lysed in cold ultrapure type I H_2O, and serial dilutions of cell lysates were cultivated in BHI plates (37ºC, 10% CO_2) for determination of intracellular numbers of bacteria. Our results indicated significant reductions in the frequencies of invasion of *pepO* and *cppA* mutants (9% and 12%, respectively), when compared to the SK36 parent strain (65%) (Mann-Whitney, $p<0.05$). These findings indicate that PepO and CppA are required for *Streptococcus sanguinis* invasion into human coronary endothelial cells. Therefore, PepO and CppA might be therapeutic targets to control streptococcal infections of the cardiovascular system. Supported by FAPESP (proc. 2018/02054-4; 2022/13074-9) and CAPES (PhD fellowship).

Keywords: *Streptococcus sanguinis,* oral cavity, cardiovascular infections, complement proteins.

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