CONTROL ID: 3223666

TITLE: Antimicrobial Activity of Omega-3 Fatty Acids Against Oral Pathogenic Bacteria

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PREFERRED PRESENTATION TYPE: Oral

SCIENTIFIC GROUPS & NETWORKS: Periodontal Research-Therapy

KEYWORDS: Biofilm, Omega-3 , Fatty Acids.

ABSTRACT BODY:

Objectives: To study the antimicrobial activity of the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in their ethyl-esters form, against oral bacteria in a subgingival *in vitro* biofilm model.

Methods: Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of EPA and DHA for six bacterial strains in planktonic state, characterizing the initial (*Streptococcus oralis* and *Actinomyces naeslundii*), early (*Veillonella parvula*), secondary (*Fusobacterium nucleatum*) and late colonizers (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) were calculated. Based on these concentrations, 72h-biofilms with the six bacterial species, were exposed for 60 secs by immersion, to EPA and DHA to study its antibacterial activity, or to different control solutions: phosphate-buffered saline (negative control), 0.2% chlorhexidine (positive control) and ethanol (solvent).
The main variable was the number of viable bacteria (colony forming units, CFU/mL), assessed by quantitative polymerase chain reaction, previously treating the samples with propidium monoazide. Besides, the biofilms were analyzed with confocal laser scanning microscopy. For each of the products, three different independent experiments, including three disks per group, were performed (n=9). ANOVA was performed with Bonferroni's *"post-hoc"* corrections for multiple comparations.

Results: EPA performed a successful reduction of, at least, one order of magnitude for all bacterial species tested. Significant reductions were observed for *P. gingivalis, F. nucleatum*and *A. naeslundii* (p<0.05). No significant impact was observed for *S. oralis*,*V. parvula*, and *A. actinomycetemcomitans*. Regarding DHA, two or more magnitude levels of reduction were observed for all bacterial species, when compared to the negative control, being those differences statistically significant (p<0.05).

Conclusions: EPA and DHA demonstrated antimicrobial activity against the tested bacterial species in *in vitro* biofilm model. Further research is needed to discern the mode of action, and to assess the feasibility of including these fatty acids in forthcoming products for biofilm control.