OMEGA 3, L. REUTERI AND VITAMIN D COLLABORATE IN DIMINISHING GUT INFLAMMATION

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Introduction:
Krill oil (KO), an extract prepared from a species of antarctic krill, Euphasia superba, containing omega-3 fatty acids, phospholipids and the natural pigment, astaxanthin, has emerged for its health benefits in treatment of inflammatory and metabolic disorders. Recently our group published a work to demonstrate its efficacy in counteracting intestinal inflammation, induced either by pro-inflammatory cytokines or by adherent-invasive Escherichia coli (AIEC) administration in vitro. Lactibacillus Reuteri is a well-documented probiotic, whose efficacy in lessening intestinal inflammation has been proven in different systems. Our aim was demonstrating that co-administration of KO, L. Reuteri and vitamin D is effective in decreasing inflammatory cytokines increase, stress fiber formation and in diminishing adhesion and invasion on AIEC, in a model of in vitro intestinal inflammation.

Methods:
Immunofluorescence
Cells exposed to the cytomix or co-exposed to cytomix and KO, L. Reuteri and vitamin D were grown at confluence and analyzed with standard fluorescence microscopy techniques for the expression of F-Actin, as markers of tissue stress.
Bacterial adhesion and invasion assays
Adhesion assay: cells were grown to confluence and infected with LF82. Adherent bacteria were recovered and plated on LB agar plates and then the colonies were counted for statistical analysis.
Invasion assay: To assess the invasiveness of LF82, CACO2 and HT29 were infected and incubated, as above. After incubation cells were incubated in gentamicin to kill extracellular bacteria. Lysis, incubation and counts were performed as in the adhesion assay.
Quantitative PCR
Expression of TNFα and IL-8 was detected by quantitative PCR. RNA integrity was checked by agarose-formaldehyde analysis.
To ensure maximum reproducibility, accuracy and statistical significance, all the experiments were carried out in triplicates.

Results
While in inflamed cells F-actin polymerization increased stress fibers, a combination of KO, L. Reuteri and vitamin D restored initial conditions. Such combination markedly reduced AIEC adhesion/invasion in epithelial cells: from 100±4,5% to 60±2,9% in CACO2 and from 100±3,8% to 39±5,2% in HT29. Finally, the same combination reduced LF82-induced mRNA expression of pro-inflammatory cytokines: TNF-alpha from 100±5,4% to 56,12 ±5,8% in CACO2 and from 100±5,8% to 51,16 ±6,2% in HT29. IL-8 from 100±5,4% to 49,5 ±5,8% in CACO2 and from 100±4,8% to 82,31 ±3,3% in HT29.

Discussion
Omega-3 and probiotics are both beneficial to the intestinal health: the advantage of using a combination of Omega-3, phospholipids and astaxanthin (KO), a probiotic (Lactobacillus Reuteri) and vitamin D is that each experimental relevant endpoint is mainly achieved by one or more of the components, so that the overall results are generally more pronounced that by using the single components separately. Our group is currently working on more endpoints to broaden the knowledge and in highlighting the overlapping and different molecular mechanisms of action.
Keywords: Omega 3, Lactobacillus Reuteri, Inflammation, Krill oil, Adhesion & invasion assay, Probiotics

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