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Anti-Rota Virus PEGylated Proteins from *Bifidobacterium adolescentis* Secretome do not Affect Viability, Tight Junction Integrity and Cytoskeleton Organization of Human Intestinal Cells - C2BBe1

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Background: Acute diarrheal disease (ADD) remains a public health problem worldwide, causing 525.000 deaths of children under 5years of age and 1.7 billion cases childhood diarrheal disease every year. Rotavirus is the most common etiological agents and causes approximately 5% of the deaths of children under 5years of age. The treatment used to control ADD produced by rotavirus is not specific and although two vaccines are available, these do not prevent the viral infection. Other important problems include lack of access in low income countries and genetic re assortment between vaccine strains and wild-type strains. As an alternative, probiotics bacteria have been used to prevent the rotavirus infection and reduce the duration and severity of diarrhea. The probiotic Bifidobacterium adolescentis produces some proteins in the extra cellular secretome which exert *in vitro* anti-rotavirus activity when they are PEGylated. The potential use of these PEGylated proteins for oral administration in humans should be established using initially *in vitro* models of human intestinal cells.

Main Goal: To evaluate the effects exerted by anti-rotavirus PEGylated proteins produced in the *B. adolescentis* extra cellular secretome on the cellular viability, tight junction integrity and cytoskeleton organization of human intestinal cells - C2BBe1.

Methods: The effect exerted on the viability of C2BBe1 cells by 14 concentrations of PEGylated proteins previously obtained from the extra cellular secretome of *B. adolescentis* was measured 24 hours post exposure by the MTT method at 540 nm. Likewise, the tight junctions (TJ) integrity of polarized C2BBe1 cells cultured in Trans Well plates after exposure to five concentrations of PEGylated proteins was monitoring daily by measuring the Trans epithelial Electrical Resistance (TEER) for 3 days. Finally, the structural stability of TJ and cytoskeleton was evaluated by immune staining and colocalization of Occludin and F-actin proteins respectively, using confocal microscopy.

Results: It was observed that none of the concentrations of PEGylated proteins evaluated was able to reduce the viability of C2BBe1cells. In contrast, cell viability increased up to 124% compared with the control without treatment (100%). Likewise, it was observed that the TEER values did not have statistically significant differences between the treatments evaluated and the negative control. On the other hand, the PEGylated proteins did not produce structural changes in the TJ and cytoskeleton of polarized C2BBe1 cells. Interestingly, there was an apparent increase in the amount of occlude in that must be confirmed.

Conclusions: Anti-rotavirus PEGylated proteins obtained from the extracellular secretome of *B. adolescentis* do not affect the structural integrity of human intestinal C2BBe1 cells. This findings predict safety for *in vivo* tests.

Keywords: *Bifidobacterium adolescentis*, secretome proteins, Acute Diarrheal Disease, anti-rotavirus.

Biography:

Juan Carlos Ulloa Rubiano is a professor and Co-ordinator of Virology Laboratory, Faculty of Sciences of the Pontificia Universidad Javeriana, Bogotá, Colombia. Dr. Ulloa has worked in virology for 17 years, mainly with viruses that produce diarrhea (Astrovirus and Rotavirus) in infants under 5 years old. The projects in which he has been involved are related to molecular virology and antivirals development from diferent sources such as probiotics bacteria and medicinal plants. In addition to scientific publications, in 2017 he patented a bioactive antiviral fraction from a medicinal plant.