Title: ANALYSIS OF THE GUT MICROBIOTA OF RATS TREATED WITH VIOLACEIN

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Abstract:

The normal microbiota plays a crucial role in the host health by acting as a barrier against invasion of pathogens and contributing with important metabolic functions. Many factors, including diet, antimicrobials and stresses can cause alterations in these populations that, in the intestinal tract, can be associated with inflammatory and allergic disease and metabolic disturbs. Chromobacterium violaceum is a Gram-negative bacterium present in the soil and water of tropical and subtropical areas and it produces a pigment called violacein that possess several functions, such as, antibacterial, antiviral, antifungal and antioxidant activities. Some riverines populations consume these contaminated waters but, nonetheless, they do not seem to develop any type of illness related with this bacterium. Thus, we decide to use an animal model to evaluate the violacein interference in the gut microbial population. To perform this experiment, we used three groups of male Wistar rats (10 weeks old). Violacein was extracted from C. violaceum, solubilized in 5% DMSO and diluted in sterile water to obtain concentrations of 50 µg/ml (Group A) e 500 µg/ml (Group B). One hundred microliters of these solutions were administered directly into the mouth of the rats twice a day for a month. The control group (Group C) received only water with 5% DMSO. Rats were sacrificed and the intestinal content was collected for DNA extraction. A portion of the v3 region of 16S rRNA was amplified by PCR using universal primers and evaluated by DGGE (50-65%). Analyses of the gels showed that the violacein interfere significantly in the gut microbiota of the rats. We also performed quantitative PCR targeting Bacteroidetes, Firmicutes, Actinobacteria and Gama-Proteobacteria. Preliminary results showed that Bacteroidetes and Gama-Proteobacteria were affected by violacein but we need to do more experiments including other phyla and, in some cases, genera as targets. Sequences obtained in 454 GS Titanium platform, demonstrated that, in the presence of violacein, the phyla Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria (Propionibacterium) and some Firmicutes were negatively affected. The opposite effect was found in some members of Firmicutes and Actinobacteria. Further analyses are crucial for a better understanding of how the violacein affects gut microbiota and whether this change would be beneficial to the host, and may thus become an alternative treatment of intestinal diseases.

Keywords: microbiota, violacein, Chromobacterium violaceum

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