THE INFLUENCE OF FOOD OLIGOSACCHARIDES (PREBIOTICS) ON ADHESION OF C. DIFFICILE TO THE HUMAN COLONIC EPITHELIAL CELL LINE (HT29/C1) IN VITRO: A PILOT STUDY.

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Background

Prebiotics are usually short-chain oligosaccharides with up to 60 polymers and are not digested by human digestive enzymes. The function of prebiotics is to provide beneficial effects to the host by stimulating the growth of selected natural microbiota bacteria. Recently, a mechanism by which oligosaccharides might interfere with bacteria has been proposed. This model is based on the observation that exogenous oligosaccharides may act as molecular receptors that can competitively inhibit bacterial adherence. Simply started, rather than binding to host mucus, bacteria bind to the soluble decay oligosaccharides and is replaced or flushed away with faeces. So far, the antiadhesive effect of those substances has been described only for few bacteria. The aim of this research is to provide new data to evaluate the in vitro potential of the food oligosaccharides for inhibiting the adhesion of C. difficile to the human epithelial cells in vitro.

Material and methods

The influence of five oligosaccharides (inulin, mannose, fructooligosaccharides (FOS), cellobiose, and raffinose) on the adhesion of 10 clinical C. difficile strains belonging to the PCR-ribotype 027 and reference C. difficile 630 to HT29/C1, was investigated. For the experiment, cells were cultured in Dulbecco’s modified Eagle medium (DMEM) with high glucose (4.5 g/ml), supplemented with L-glutamine and antibacterial/antimycotic solution. All cells were grown in 75 cm² flasks and incubated at 37°C with 5% CO₂ and 95% relative humidity. After reaching 70-80% confluence, cells were split and cultured as above in 24-well plates with fresh media that contained no antibiotic/antimycotic solution. For the experiment cells were washed twice by PBS and fresh medium containing 1% final oligosaccharide concentration was added and incubated for 4h. Than inoculum was added to every well and incubated for one more hour under anaerobic conditions and 37°C. Negative control were cells incubated with bacteria, without oligosaccharides. After incubation cells were trypsinized, the contents of all wells were diluted and blood Columbia Agar plates were inoculated by 20µl of dilution in duplicate. Plates were incubated for 48h, at 37°C under anaerobic conditions. The grown colonies were counted. This experiment was performed three times for each strain. Averages and percentage of adhesion were calculated. Data were tested using one-way ANOVA and Tukey’s HSD post-hoc analysis.

Results

Most of examined oligosaccharides displayed anti-adhesion properties (Fig. 1). Mannose decreased adhesion of C. difficile to the colonic epithelial cells to 54.4% (p<0.001), FOS and raffinose to 70.2% (p<0.05) and 72.6% (p=0.063) respectively; cellobiose presented weak anti-adhesion properties (92.5%, p=0.96). It is interesting that inulin, often used for the supplementation of diet, increased the adhesion of C. difficile to HT29/C1 in vitro (108.1%, p = 0.97).

Conclusions

We demonstrated that all prebiotics except inulin, were anti-adhesive for C. difficile strains binding to human HT29/C1 cells. Inulin may increase C. difficile adhesion, which may have an adverse effect when using for prophylactic purposes in CDI patients with CDI. Further studies evaluating the anti-adhesive properties of food oligosaccharides on C. difficile are urgently needed.

This work was supported by the National Science Centre, Poland (Grant number: 2017/25/N/NZ6/01763)