Carrageenan intake affects the gut microbiota composition and the epithelial permeability

Main author: Teresa Requena (Spanish National Research Council (CSIC))

Co-authors: Alicia Bellanco, Carmen Peláez, M. Carmen Martínez-Cuesta

INTRODUCTION

Diet has a profound impact on gut microbiota composition and metabolic activity, which in turn can affect health. Recent epidemiological studies have linked the increased intake of highly processed foods and beverages, as well as some food additives and processing aids that are used in their production, with non-transmissible chronic diseases eventually associated with the so-called industrialised microbiota. Therefore, it seems appropriate to systematically evaluate the safety of food additives at the estimated dietary exposure levels including the human microbiome in the risk assessment. Thus, the aim of this work was to determine the possible impact of carrageenan, a food additive composed of a mixture of high molecular weight sulphated polysaccharides, on intestinal microbiota, and this in turn, on the intestinal barrier function.

METHODOLOGY

A four-stage reactor system (Simulator BFBL) designed to simulate, in vitro, the small intestine and the microbial conditions of the three regions of the human colon was used in the study. The sulphated mucopolysaccharide extracted from red algae carrageenan (E-407), containing predominantly κ-carrageenan (one sulphate per disaccharide unit) and lesser amounts of λ-carrageenan (three sulphates), was added at increased doses (54, 180, 360 and 540 mg/day) to a pool of child faecal microbiota stabilised at steady-state condition in the Simulator BFBL. Then, each carrageenan dose was distributed over three daily intakes over 7 days. Samples were collected daily from the three colon reactors and centrifuged. The microbiota composition was analysed in the pellets by 16S rDNA amplicon-based metagenomics. In addition, both pellets and supernatants were used to analyse their effect on the integrity of Caco-2 epithelial monolayer, determined by measuring the Transepithelial Electrical Resistance and the paracellular permeability by using the fluorescent colorant Lucifer Yellow. The cellular viability of these cells was assayed using the reduction of MTT.

RESULTS

The data showed a dose-response effect of carrageenan intake that caused a significant increase of permeabilising and proinflammatory bacteria such as Desulfovibrio desulfuricans (Spearman's correlation index, rs = 0.939**), Ruminococcus torques (rs = 0.940**), Eisenbergiella (rs = 0.957**) and Allisonella (rs = 0.896**). Moreover, a significant decrease of butyrate-producers such as Fusicatenibacter (rs = -0.700**) was also observed.

The carrageenan did not have any impact either on cellular viability or on the epithelial monolayer integrity. However, when hydrolysed carrageenan (heated at 121 °C for 15 min at pH < 2) was tested, the cellular viability of Caco-2 cells dropped and the monolayer permeability increased. Furthermore, a dose-response decrease of the integrity of the cellular monolayer and an increase of the paracellular permeability were observed when supernatants of carrageenan-fed-microbiota were assayed, thus indicating the potential hydrolysis of carrageenan by the gut microbiota. In addition, the carrageenan-fed microbiota decreased the monolayer integrity of Caco-2 cells and increased the paracellular permeability at the highest carrageenan doses tested.

DISCUSSION

The safety of food-grade carrageenan (E 407) is based on the assumption that it is not hydrolysed through its passage along the human gastrointestinal tract or degraded by the gut microbiota. However, our results indicate that carrageenan intake at doses representing the estimated dietary exposure levels in children may negatively modify gut microbiota composition, promoting the growth of proinflammatory microbiota. Also, these changes could be associated with the capability of some microbial groups to hydrolyse carrageenan, which in turn would have an impact on the intestinal barrier function.

Based on these findings, additional carrageenan risk assessment analyses should determine the physiological relevance of the in vitro observed results. Furthermore, the effect of the food matrix where the additive is added should be taken into account.