INTRODUCTION

There is an accumulating evidence that gut microbiota play an important role in the induction and maintenance of various allergic, autoimmune, metabolic diseases and cancer [1,2,3]. The ratio of beneficial (anti-inflammatory) versus harmful (pro-inflammatory) microbiota seems to play a central role [4]. The composition of gut microbiota is influenced by genetic and environmental factors. The single most important environmental factor is diet [5].

A preservative is naturally occurring or synthetically produced substance that is added to food to prevent microbial growth or undesirable chemical changes. Preservative food additives can be used alone or in combination with other methods of food preservation. Preservatives may be antimicrobial preservatives, which inhibit the growth of bacteria or fungi, including mold, or antioxidants, which inhibit the oxidation of food constituents. Common antimicrobial additives include sorbic acid and its salts, benzoic acid and its salts, potassium sorbate, calcium propionate, sodium nitrite, sulfites and disodium EDTA.

AIMS

The aim of the project is to evaluate whether and to which extent commonly used food additives modify the composition of gut microbiota.

- **In vitro part**: to test inhibitory effect of widely used antimicrobial food additives on single bacteria isolated from human gut microbiota
- **In vivo part**: to test the influence of antimicrobial food additives on the composition of human gut microbiota (experiments with germ-free mice colonized with human gut microbiota)

METHODS

We have isolated six aerobic bacteria from human fecal sample. We have grown aerobic bacteria in a tryptic soya broth to OD600 1.0 and incubated them, for 24 hours at concentration of 10^3 bacteria per well, with antimicrobial food additives, namely sodium benzoate, sodium nitrite, potassium sorbate, and sodium sulfite, at different concentrations. Then minimal inhibitory concentrations (MIC) and fractional inhibitory concentration (FIC) were determined using spectrofotometry.

To address in vivo experiments we colonized germ-free C57BL/6 mice with the microbial community sample from a healthy adult human. Experimental mice were treated with selected food additives supplemented in drinking water. The composition of gut microbiota was identified using bacterial community analysis (QIIME) of sequenced genomic DNA from fecal samples.
Results

The MIC of sodium benzoate, potassium sorbate and sodium nitrite is different for particular AMFAs and isolated aerobic human gut bacteria, MIC of sodium sulfite is the same for each of them and much higher concentrations are needed to inhibit growth. The FIC measurements show that sodium benzoate and sodium nitrite have a synergistic effect on Enterobacter, one strain of Klebsiella and one strain of E.coli. Sodium benzoate and potassium sorbate have a synergistic effect on Enterobacter, Klebsiella, and one strain of E.coli. Sodium nitrite and potassium sorbate have a synergistic effect on Enterobacter, Klebsiella, and one strain of E.coli. The effect of sodium sulfite was significantly different from other AMFAs.

When tested the effect of the presence of AMFA in diet in vivo, we have found that low AMFA concentrations decrease the diversity of human gut microbiota, specifically the Actinobacteria and Verrucomicrobia phylum disappear, and increase the representation of the Proteobacteria Phylum, high AMFA concentrations in diet entirely disrupt the gut microbiota ecosystem with the Proteobacteria phylum overgrowth.

Discussion

We have tested the inhibitory effect of four common AMFAs on the growth of culturable gut bacteria. We have found that this effect is different for each bacteria and AMFA and can be synergistic in some conditions, so we hypothesize, that the presence of many different AMFAs in diet can influence the growth of single gut bacteria and contribute to dysbiosis, albeit most of gut bacteria are anaerobic and unculturable. The effect of sodium sulfite was different from the others, it seems that it can react with others AMFAs to prevent its acting.

The in vivo experiments were performed with human gut microbiota from one donor, so we can not exclude the resulting effect of AMFAs in diet on the composition of gut microbiota is universal.

Conclusions/Summary

To study the effect of preservatives on the composition of human gut microbiota we performed in vitro studies and for in vivo experiments we exploit a humanized mice model. In vitro, AMFAs inhibit the growth of human gut bacteria in a different manner and in some concentrations, the growth inhibitory effect is synergistic.

Our preliminary data from microbiome profiling of additive-treated mice colonized with human gut microbiota show that antimicrobial food additives have the capacity to alter the composition of gut microbiota.

We have found, that the AMFAs present in diet have the potential to influence the composition of human gut microbiota. Further work is need to ascertain the impact of changed microbiota on the function of immune system and the development of disease.

References

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