Streszczenie w języku angielskim

THE ROLE OF METHANOGENIC ARCHAEA IN THE INFLAMMATORY BOWEL DISEASE IN CHILDREN

Introduction: Methanogenic archaea are an important component of the intestinal microbiota, and their colonization of the human intestines begins in early ontogeny. Changes in the composition of methanogenic archaea and/or their numbers may be related to the development of various diseases, including inflammatory bowel disease (IBD). Bacterial dysbiosis is a known phenomenon associated with the development of IBD in children and adults, but little is known about the role of archaeal dysbiosis, particularly in children. The importance of methanogenic archaea in IBD was presented in a review article entitled: ***The Role of Methanogenic Archaea in Inflammatory Bowel Disease—A Review*** *(Cisek et al., Journal of Personalized Medicine, 2024).*

Objectives: The main aim of the doctoral dissertation was to assess the importance of methanogenic archaea in children with IBD. It was achieved by implementing detailed objectives that were described in individual original articles. Detailed objectives included: 1) development of an original protocol for the detection of methanogenic archaea in fecal samples, validation of the protocol using chicken droppings samples; 2) assessment of the impact of environmental factors on the population of methanogens in the chicken intestines in preclinical studies; 3) analysis of the number of methanogenic archaea in the feces of children with IBD compared to children from the control group, and assessment of the relationship between the number of these microorganisms and the type and degree of disease activity and the age of the children.

Research groups, materials and methods: The study involved 124 patients aged 3 to 18 years, including 45 children diagnosed with Crohn's disease (CD), 52 children diagnosed with ulcerative colitis (UC), and 27 children from the control group. This group consisted of children without a diagnosis of IBD. The activity of the disease was assessed using the following indices: the PUCAI index (Pediatric ulcerative colitis activity index) and the PCDAI index (Pediatric Crohn's disease activity index) and the assessment of fecal calprotectin (FCP) concentration. The FCP concentration was determined using chemiluminescence technology. Preclinical studies included 174 chickens bred in three breeding systems (free-range, farm and experimental), differing in terms of access to the natural environment, antibiotic supplementation and diet. The influence of age and the type of sample (stool vs cecal content) on the abundance of methanogens was also examined. DNA was isolated from feces of patients, droppings and cecal contents collected from chickens. Additionally, DNA samples from 3 species of methanogenic archaea and one plasmid construct, as well as DNA samples isolated from 21 bacterial strains, were used as controls. Quantitative assessment of methanogen populations in the intestines of humans and animals was performed using the real-time PCR technique, targeting, among others, a

methanogen-specific *mcrA* gene, i.e. the gene encoding an enzyme involved in the last stage of methanogenesis occurring in these microorganisms.

Results: The results were presented in 3 original articles. The work entitled ***Improved Quantitative Real-Time PCR Protocol for Detection and Quantification of Methanogenic Archaea in Stool Samples*** *(Cisek et al., Microorganisms, 2023)* described a development of protocol for the detection of methanogenic archaea with the use of *mcrA* gene. It resulted in better validation parameters than the previous, commonly used method. It was characterized by increased specificity and sensitivity, reproducibility and a wider linear detection range (as it allowed for determination of 7 instead of 6 orders of magnitude of the *mcrA* gene copies). Therefore, the author's protocol allowed to reduce the number of methanogen cells (genomes) in the tested material necessary to obtain a positive result by as much as one order of magnitude (e.g. from 571 down to 57 copies of the *mcrA* gene copies of the *Methanomicrobium mobile* species in reaction mixture). The lowest quantifiable copy number of the *mcrA* gene at a frequency of 100% was 21 copies per reaction. The new protocol also reduced the risk of false positive results in samples not containing methanogens. The developed protocol allowed to minimize the negative impact of primer dimerization and other cross-reactions (the source of which may be bacterial DNA isolated from stool) on the real-time PCR results. Moreover, when the new protocol was used, the presence of methanogens was detected in all 20 droppings samples tested, while as many as 7 samples tested negative when using the protocol of other authors.

The conducted preclinical studies described in the article entitled ***Microorganisms Involved in Hydrogen Sink in the Gastrointestinal Tract of Chickens*** *(Cisek et al., International Journal of Molecular Sciences, 2023)* confirmed the effectiveness of the developed methodology. The study showed that in a group of experimental chickens bred in strictly controlled conditions, isolated from the natural environment, the methanogenic archaea were not detected, while the cecum of the free-range and farm chickens was inhabited at a similar level, i.e. 10^4 to 10^5 cells/gram of the tested sample. Moreover, it was shown that the number of methanogens varies depending on the type of sample (droppings contained significantly more methanogens than intestinal content; $p<0.001$), and on the age of the animal (in the group of 1-week-old farm chickens, significantly fewer methanogens were observed than in older farm chickens at 3-4 and 5-6 weeks of life; $p<0.01$). It was therefore shown that environmental factors, the age of the chicken and the type of sample collected for testing were important factors determining the presence of methanogenic archaea in the tested material.

The work entitled ***Methanogenic Archaea in the Pediatric Inflammatory Bowel Disease in Relation to Disease Type and Activity*** *(Cisek i wsp., International Journal of Molecular Sciences, 2024)* demonstrated that in children suffering from IBD, a statistically significant decrease was observed in the number of the total population of methanogens, both in the stool of children suffering from UC ($p<0.001$) and CD ($p<0.05$), compared to children from the control group. Moreover, the abundance of

methanogens was lower in UC than in CD ($p<0.05$). The prevalence of these microorganisms was also significantly lower, especially in the group of patients with UC ($p<0.05$) where it was 83% compared to the control group, in which the prevalence of the total population of methanogens was 100%. In the same group of patients, a positive correlation was also found between the number of *Methanosphaera stadtmanae* and the concentration of fecal calprotectin ($Rs = 0.41$; $p<0.05$). On the other hand, in the group of patients with active form of CD, a statistically significant decrease in the number of total methanogens ($Rs = -0.56$; $p<0.01$) and *Methanobrevibacter smithii* was observed with age ($Rs = -0.53$; $p<0.05$).

Summary of the results and conclusions: The study showed that 1. the abundance and prevalence of methanogenic archaea in feces change depending on environmental factors and the age of the host; 2. Archaeal dysbiosis occurs in children with IBD, and it depends on the form of IBD (CD vs UC) and the activity of the disease; 3. The results obtained and the literature review demonstrate that archaeal dysbiosis may be a consequence of the inflammatory process accompanying IBD, and not a disease causing factor.