Szakmai zárójelentés

A vastagbél epithelialis barrier és mucus közötti interakció szerepe az irritábilis bél szindróma patogenezisében

Final report

Study of the interactions between colonic epithelial barrier and mucus in the pathogenesis of irritable bowel syndrome

OTKA-2015 ID: 116370

Irritable Bowel Syndrome (IBS) has a great clinical significance, as more than 10 % of the general population is suffering from symptoms of this disease. Despite of its epidemiological significance the pathophysiology of IBS is still not completely understood. In our study, we aimed to investigate the role of the intestinal permeability and colonic mucus layer in the pathogenesis of the disease. Examination of this issue is a significant challenge, as the intestinal mucus layer is extremely vulnerable.

As a first step we introduced a novel technique in the Hungarian human research for measuring colonic transepithelial permeability using Ussing-chambers system. In addition to the ex vivo functional experiments histological methodologies were set up. To assess colonic mucus thickness, we used mucus staining and measured the surface mucus layer through microscopic techniques in fixed biopsy samples. We counted mucus producing MUC-2 positive cell counts. To measure bacterial penetrance in the mucus layer, we adjusted an immunohistochemical method. Universal bacterial probe (FISH) was used to detect bacteria in the mucus layer. We set up the method to measure the thickness of the bacterially impermeable mucus layer in colonic biopsy samples.

In parallel with the innovative methodology preset, we started to recruit patients for the study. Preliminary study was carried out to investigate the colonic mucosal MUC-2 expression in patients with diarrhoea predominant IBS (IBS-D). Due to the unexpected length of a public procurement of Ussing chamber system the beginning of the real study period was postponed. Therefore, to increase the scientific value of the project, the originally planned basic pathophysiological experiments were supplemented with a therapeutic arm. We planned to assess the efficacy of a 3-month therapy of a novel mucoprotectant medication (xyloglucan) in IBS-D. Xyloglucan is a branched polysaccharide consisting of a cellulose-like backbone that carries xylose and galactosyl-xylose substituents. This molecular structure can form a mucin-like structure. The chemical properties of xyloglucan help in biological structures to block the

bacterial biofilm, to protect tight junctions (examined in Caco cell lines) and can block bacterial invasion. Therapeutic effect of xyloglucan has been already tested in airway allergy, in recurrent urinary infections, in conjunctive tissue and skin regeneration of the eye, and lately intestinal application has been also investigated. Based on preliminary clinical study xyloglucan is safe and effective in human use to reduce stool number and pain in IBS-D patients. The mechanism of action in the intestine is not scientifically demonstrated yet. The novel therapeutic arm of the study consists of the following elements. Symptoms and psychological questionnaires were registered before, during and after the therapy. Baseline colonoscopy was performed before the treatment, and colonoscopic sampling was repeated after the 3-month therapeutic period. Our novel aim was to measure the effect of the mucoprotectant agent on the mucus thickness, permeability and symptoms. We succeeded to recruit and initially examine 5 patients, and treat them with the mucoprotectant product. 4 patients could complete the project with the closing colonoscopy. At this point, on March 16, 2020 COVID-19 pandemic blocked the further recruitments and examination of the patients. Considering that an appropriate control group is required for the originally planned pathophysiological study, data of the first 5 patients were not sufficient to design the age and sex matched control study group. Therefore, we could not involve any control subject at the time of discontinuation of elective endoscopic procedures.

Results

1. Preliminary study of colonic mucosal MUC-2 expression

6 control patients (3 male/3 female, age 48.6 ± 6.32 years) and 14 IBS-D patients (8 male/6 female, age: 48.2 ± 2.49 years) were recruited. All the patients went through a colonoscopy;

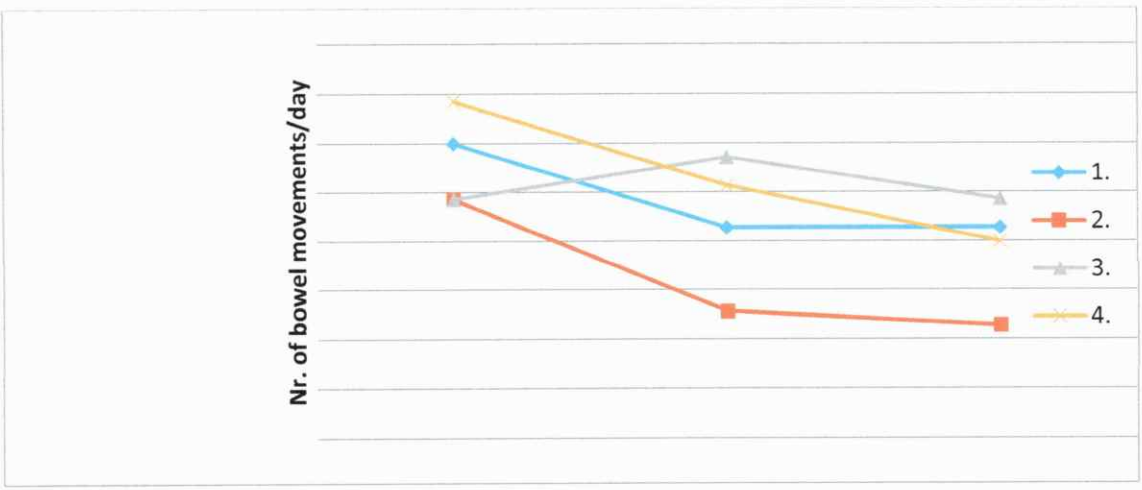
biopsies were taken from the sigmoid colon. Immunohistochemistry was performed to visualise MUC-2 mucosal expression. Microscopic analysis and quantification was made by counting MUC-2 positive epithelial cells in relation to total epithelial cell count. Colonic mucosal MUC-2 expression was significantly higher in IBS-D patients compared to the controls. (0.34 ± 0.009 vs. 0.42 ± 0.018, $p=0,01$).

2. Basic study planned in the application extended with therapeutic arm (novel mucoprotectant drug)

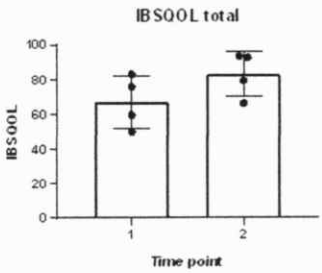
WE could recruit 4 IBS-D patients, who went through the 3-month xyloglucan treatment.

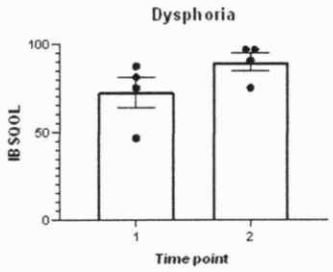
Nr. of patient	Sex	Age (years)
1.	♂	22
2.	♂	49
3.	♀	24
4.	♀	32

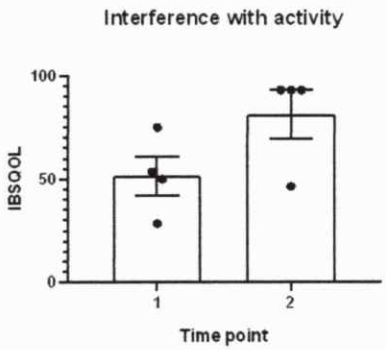
All participants reported improvement of diarrhoea.

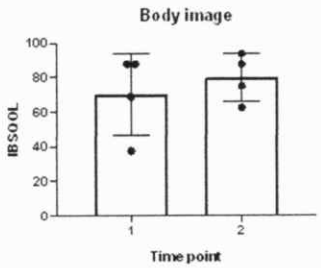


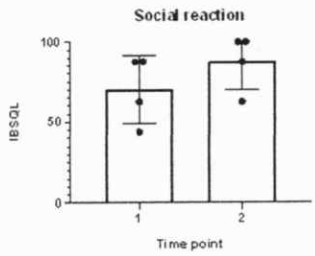
Questionnaires revealed improvement in quality of life after 3-month xyloglucan treatment.

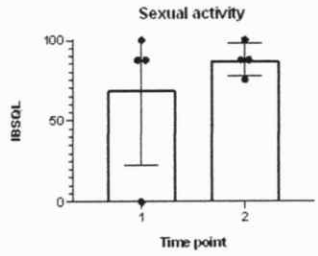


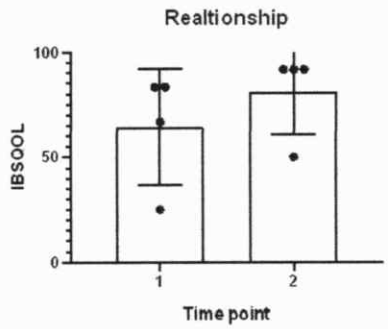




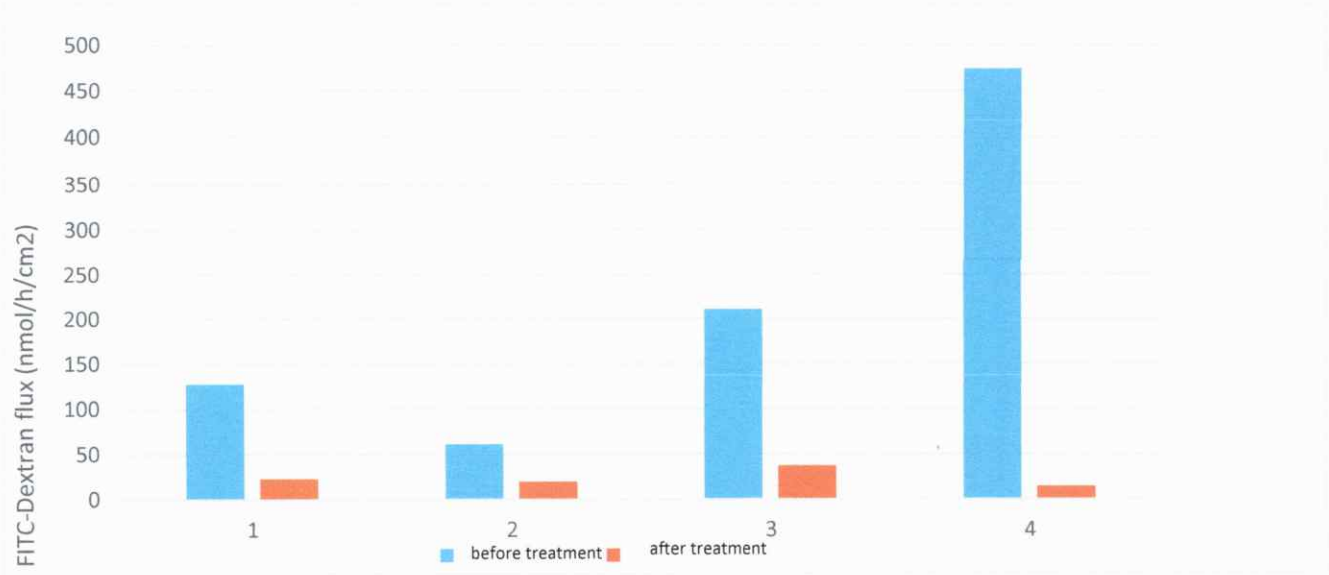






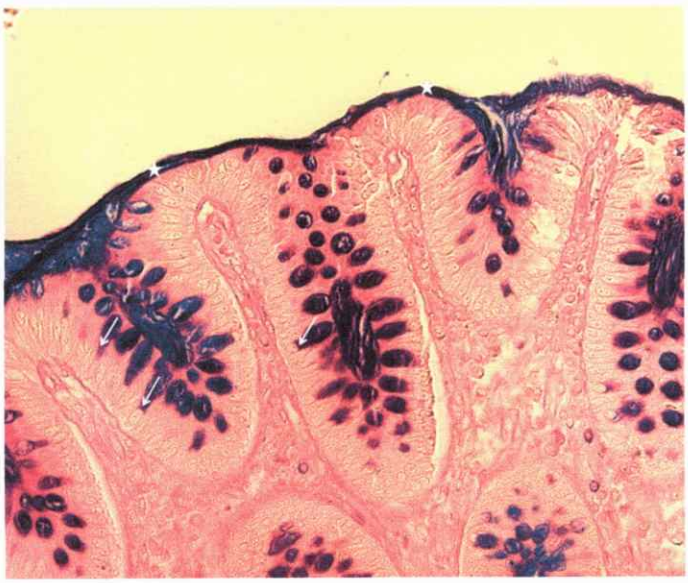


Ex vivo permeability was measured by Ussing Chambers system in samples from the ascending colon. Our results show a reduced intestinal permeability after xyloglucan treatment.

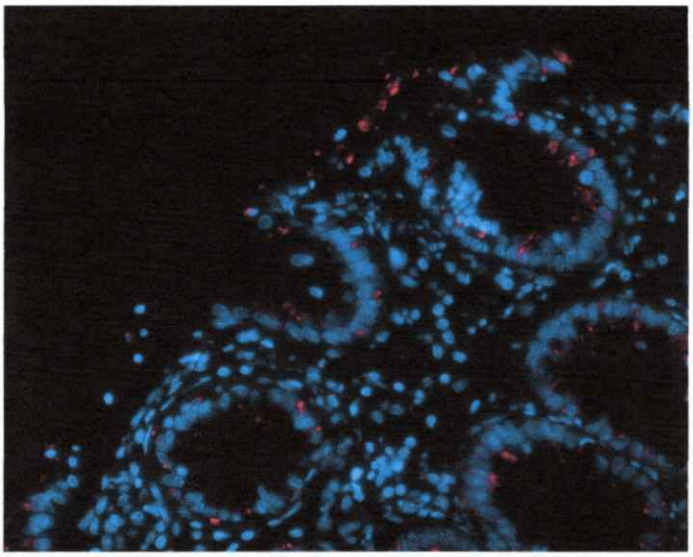


Mucus thickness, MUC-2 positive cells staining, and mucus bacterial penetrance staining was technically successful, but the poor quantity of data did not provide sufficient information for data analysis.

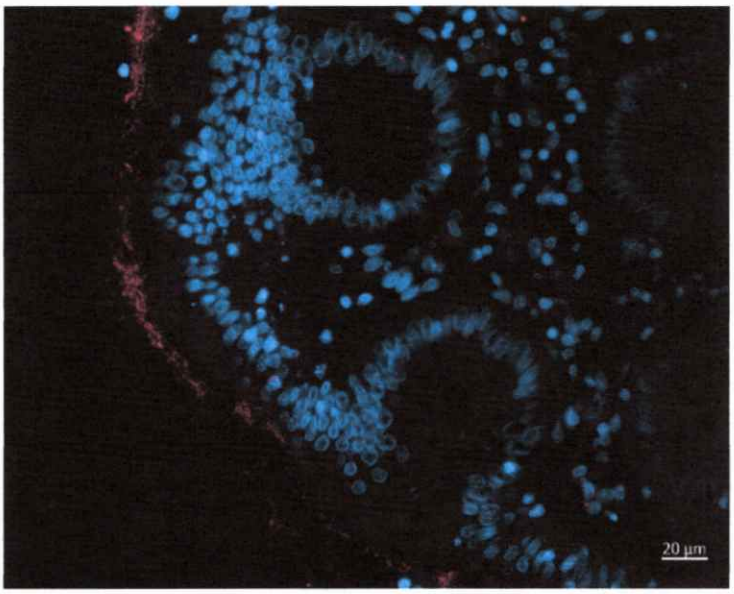
1. Mucus thickness



2. MUC-2 immunostaining



3. Mucus bacterial penetrance measurement results in the ascending colon



Data missing to complete the publication

Our data are not sufficient for publication, however the methodology and project structure was a basis of a BSc Thesis in the University of Szeged, Faculty of Biology. We continue the project,

and complete the patient's recruitments to have sufficient data for the publication after the COVID-19 pandemic induced restrictions will be suspended. Optimally, involvement of 6 IBS-D patients and 10 control subjects will be sufficient for publication in a valuable journal. Examination of constipated IBS patients is not essential for the first article.

Reasons for the deviations from the original study design and for the insufficient publication activity

The reason for the first deviation from the research plan was the delayed purchase of the Ussing chamber system due to the delay in the procurement procedure. The procurement contract was signed on September 21, 2018, and the Ussing system was installed on November 11, 2018.

Further deviation was caused by COVID epidemic. Since March 16, 2020 the Hungarian government has suspended the non-emergency patient care and in parallel the Hungarian Gastroenterological Association has restricted the endoscopic examinations to the emergency level. The latter regulation is in force at the time of this report. Then from November the gastroenterological department involved in the research project became a COVID unit. Since the focus of the project is a benign, functional gastrointestinal disorder, due to the COVID situation, colonoscopies in IBS patients have not been performed since the onset of the epidemic. Based on the epidemiological situation, continuation of the project is unpredictable at the time of this report.

Final Conclusions

Based on our preliminary results colonic MUC-2 expression and permeability changes are present in IBS-D patients. Xyloglucan seems to reduce diarrhoea and improve the quality of life in this patients. The treatment reduces the colonic permeability. These data are promising, as the health emergency allows, the project participants will make every effort to continue and successfully complete the research.

Szeged, March 4, 2021

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