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Background: Dysfunctions of intestinal barriers are reported to play important roles in the pathogenesis of Ulcerative colitis (UC). Cellular component of intestinal barriers is intestinal epitherial cells (IECs). Thus, homeostasis of IECs would be an object to prevent inflammatory bowel disease. Transforming growth factor (TGF)-β plays an important role in maintaining homeostasis of IECs. Calcineurin inhibitors are reported to upregulate TGF-β signaling pathway in renal tissue. Tacrolimus (Tac) is highly effective for steroid-resistant UC. The immunosuppressive function of Tac was reported to inhibit T cell proliferation and induce apoptosis of activated T cells. In addition, it is reported that Tac also suppressed the activity of macrophage and induced apoptosis. However, the effects on IECs have not clarified yet. The aim of this study was to investigate the effects of Tac on TGF-β signal in IECs and to examine its protective effect in an experimental colitis model.

Methods: Colitis was induced by feeding of 4% dextran sodium sulfate (DSS) in C57/BL6 mice. We investigated protective effects of Tac with or without anti-TGF-β antibody by measuring body weight, histological assessment of mucosal damages and TdT-mediated dUTP nick end labeling (TUNEL) analysis for apoptosis of IECs. Next, we assessed phosphorylation of Smad2/3 in purified IECs by western blotting (WB) and muptiplex bead assay (MBA). Expression of TGF-β and TGF-β receptors in colonic epithelial cells were assessed by MBA and WB. We also examined whether Tac has direct effects on intestinal epitherial cells (IECs) or not using the intestinal epithelial cell line Caco2. The expression of TGF-β receptor type II (TGF-βRII) mRNA was evaluated by quantitative polymerase chain reaction (qPCR).

Results: Treatment with Tac ameliorated mucosal destruction and prevented from weight loss through the reduction of epithelial apoptosis. The protective effect of Tac was partially maintained under anti-TGF-β antibody treatment. The expressions of TGF-βRI and II in purified IECs from Tac-treated mice were upregulated compared with that in vehicle-treated mice. Phospho-Smad2/3 expression at 6 hours after Tac injection was upregulated and the effect was observed despite of anti-TGF-β treatment. The expressions of active TGF-β1, 2, 3 in IECs or lamina propria in Tac-treated mice were not upregulated compared with that in vehicle-treated mice. In Caco2 cells, the expressions of TGF-βRI and II were upregulated in the treatment with Tac.

Conclusions: These results indicate that Tac has an protective effect from apoptosis-mediated epithelial injury via activating TGF-βR-Smad pathway.

P-300

Effects of Nutrients on Miniguts Growth

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Background: The intestinal tract performs a duo role of absorbing substances for the body and protecting the body from the outside world. The intestinal epithelium is the part of the tract that absorbs nutrients through its large surface area. Since the intestinal epithelium is constantly bombarded with different chemicals and nutrients in performing its function, it is critical to determine what kind of nutrients enhance or inhibit intestinal health and functionalities. Miniguts derived from intestinal stem cells provide a new dimension to research and observe the way organs work. In this study, we conducted multiple tests of various nutrients on gut functionality using mice intestinal organoids as the model. Significant results observed were caffeic acid inhibited organoid growth in a concentration-dependent manner, curcumin exhibited a range of effectiveness, and vitamin C did not affect organoid growth.

Methods: Because the major function of the intestinal tract is to absorb nutrients, liquids, and other substances for the body, it is important to understand the effects of ingesting different food nutrients on the gut. We utilized mice intestinal organoids to model a functional gut system. In this study, we aimed to determine the effects of the different food nutrients (MSG, vitamin C, chlorogenic acid, caffeic acid, curcumin, and mHPP) on gut function. The mice intestinal organoids were grown in 24 wells in 2 plates for 7 days without adding any nutrients. Pictures of the organoids were taken by the LAS in ×5 and ×10 magnification every other day. After growing for 7 days, food nutrients were added to the wells in 100, 300, and 600 μg/mL solutions. A group of organoids were not given any nutrients to be the control. After administering nutrients, pictures of the organoids were taken daily with the LAS software in ×5 and ×10 magnification. The surface areas of the organoids were calculated using ImageJ and graphed.

Results: In this experiment, we observed the effects of various food nutrients. Curcumin exhibited a range around 300 μg/mL that stimulated organoid growth. This shows potential in cancer treatments and recoveries. Caffeic acid inhibited growth in higher concentrations, while vitamin C did not strongly affect organoid growth. mHPP inhibited growth slightly, but it is possible that it affects cells in other ways.

Conclusions: Future work would be to find out how the nutrients affect the cells in ways that amplify or inhibit their growths. Other future work would be to observe the effects of MSG again because its results did not comply with previous studies. While chlorogenic acid exerted a slight influence on positive organoid growth in the 300 μg/mL concentration, there were not many significant impacts and should be observed again.

P-301

High Concentration Multistrain Probiotic Produced at Different Manufacturing Sites: Comparative Analysis

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Background: Due to the live nature of probiotics, changes in the manufacturing processes or facilities can result in differences in the product itself (Sanders et al, *Ann N Y Acad Sci.* 2014;1309:1–18). Recently, under the brand VSL#3, a formulation produced by a manufacturer different from the previous one, has been commercialized in some European Countries. We compared the VSL#3 produced in USA with VSL#3 produced in Italy and found that the biological effects of the 2 products when compared in vitro on tumor cell lines' viability, proliferation, cell cycle profile and apoptotic death levels were significantly different (Cinque et al, *PLoS One*. 2016, In press). Here we report additional data regarding the physical, chemical and biological characteristics of the 2 formulations available in Europe.

Methods: Samples of VSL#3 were biophysico-chemically characterized from their solid state by differential scanning calorimetry and thermogravimetry analysis and for their kinetics of stability, cell size (hydrodynamic diameter) and zeta potential (electrophoretic mobility) in aqueous dispersion. The live/dead status of bacteria in the VSL#3 product was assessed using a mixture of SYTO®13 green fluorescent nucleic acid stain and the red fluorescent nucleic acid stain, propidium iodide. Cells were inspected visually using an epifluorescence microscope. Stocks of 1 g (10^2 billion bacteria) of each VSL#3 lot were suspended in 10 mL culture medium and incubated in a thermomixer, with shaking at 37°C for 2 hours. Intestinal epithelial (IEC-6) cells were plated onto polylysine coated coverslips. Thirty thousand cells were incubated in the presence or absence of bacterial suspension (1000 live bacterial cells/cell) for 24 hours. Afterward the cells were observed using a light microscopy or prepared for scanning electron microscope observation.

Results: Both powder samples showed similar melting characteristics with Tm around 116°C but slight enthalpy difference. Their decomposition/degradation were different; the USA product decomposed/degraded faster than the Italy product with temperature increase in the range of 170 to 310°C. In aqueous dispersion, the kinetics of aggregation/sedimentation as well as cell hydrodynamic diameters appeared different. The USA product exhibited a larger hydrodynamic average size and aggregated/decanted faster than the Italy product. Cell biological behavior information showed a low number (16%) of viable bacterial cells in the Italy product compared to the USA product (76%), both products at 8 months shelf life. Morphological differences between IEC-6 cell cultures treated with both VSL#3 samples were also evidenced through optical and scanning electron microscopy.

Conclusions: In conclusion, the VSL#3 made in Italy is quite different from the VSL#3 made in USA. Different biophysico-chemical characteristics and reduced number of viable bacterial cells impact on the biological profile of the product.

P-302

Villous Length Does Not Explain Decreased Expression of Xenobiotic Genes in the Small Intestine of Children with Crohn's Disease

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Background: Previous studies have demonstrated decreased expression of genes important to xenobiotic and drug metabolism (e.g., PXR, villin, CYP3A4) in inflamed versus non-inflamed intestinal tissue in patients with Crohn's disease. Interpretation of these results is confounded by lack of knowledge regarding the influence of decreased epithelial mass versus inflammation/disease, per se, on intestinal gene expression. The aim of this prospective investigation was to assess the relationship between PXR, villin, and CYP3A4 intestinal gene expression and intestinal villous length in inflamed versus non-inflamed small bowel tissue in children with and without Crohn's disease.

Methods: In addition to standard-of-medical-care clinical and histopathology review, fresh flash frozen duodenal and terminal ileal (TI) mucosal biopsy tissues from 22 treatment-naïve children with Crohn's disease, and 20 age-, sex-matched controls without inflammatory bowel disease, were assessed for inflammation extent and villous length by 2 independent experienced pediatric pathologists. Villous length ($n = 42$ children) and mRNA expression of PXR, villin, and CYP3A4, run in triplicate