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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 epithelial 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. Tacrolium (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 C57BL/6 mice. We investigated protective effects of Tac with or without anti-TGF-β antibody by measuring body weight, histological assessment of mucosal damage 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 multiplex bead assay (MBA). Expression of TGF-β and TGF-β receptors in colonoscopic epithelial cells was estimated by MBA and WB. We also examined whether Tac has direct effects on intestinal epithelial cells (IECs) or not using the intestinal epithelial cell line Cosc2. 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-βRII 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-βRI, II, and TGF-βRII mRNA in propria in Tac-treated mice were not upregulated compared with that in vehicle-treated mice. In Cosc2 cells, the expressions of TGF-βRII 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-β-Smad pathway.

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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 not in agreement (Cai 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 biophysi-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 the bacterial 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 (10^12 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 on coverslips and 96 wells plate, and 10^5 cells/plate. After 24 hours, the 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 microscope 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/d canned 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 cells cultured 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 products.