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ORAL PRESENTATIONS: DIETARY INTERVENTIONS INCLUDING PROBIOTICS, PREBIOTICS AND SYNBIOTICS

1 | Bifidobacterium Breve NCFB 2258 stimulates vagal nerve firing across an intact colonic barrier

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Objective: Mounting evidence implicates the vagus nerve in signalling between colonic bacteria and the central nervous system (CNS), in what has been termed the microbiome-gut-brain axis. However, the mechanism by which bacteria signal across an intact barrier to their eukaryotic hosts is not understood. Bifidobacterium Breve NCFB 2258 is a commensal bacterial strain which produces polyunsaturated fatty acids (PUFAs) with reported health-promoting effects. The study aim was to investigate if this bacterial strain could signal across the gut barrier to stimulate the host nervous system.

Methods: Using ex-vivo Sprague Dawley rat colonic tissue, immunofluorescent staining and calcium imaging were utilised to investigate activation of submucosal neurons in response to mucosal application of PUFA-producing probiotic secretions (supernatants). To determine if the effects were local to the enteric nervous system of if they also stimulated colonic afferents, extracellular recordings of vagal nerve activity were also undertaken.

Results: Mucosal exposure to the bacterial supernatants stimulated increased nuclear expression of cFos and peroxisome proliferator-activated receptor alpha (PPARα) in submucosal neurons. A robust increase in neuronal $[Ca^{2+}]i$ was also observed in response to mucosal application of supernatants. This response was reduced ($P<.001$) but not abolished by the PPARα antagonist, GW6471. Similarly, exposure of the colonic mucosa to supernatants ($P<.001$) stimulated increased firing in vagal afferents. The PPARα antagonist reduced ($P<.001$) but did not abolish this response.

Conclusions: These findings illustrate that PUFA secretions from Bifidobacterium breve NCFB 2258 signal across a healthy intact gut barrier to the intrinsic and extrinsic gut nerves. Supernatants induced activation of underlying submucosal neurons, which regulate absorption and secretion, but may also act as a relay for gut-to-brain signalling. Indeed, supernatants also increased vagal afferent firing, which was mediated in part by activation of PPARα. These findings begin to elucidate the molecular mechanisms underlying signalling by specific bacteria in the microbiome-gut-brain signalling axis.

Policy of full disclosure: None.

2 | Protease activity and tryptase expression is increased in a post-inflammatory rat model for visceral hypersensitivity

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Objective: Previously, we confirmed beneficial effects of the serine protease inhibitors nafamostat mesilate and the newly developed UAMC-0050 and UAMC-1162 on visceral hypersensitivity in an acute and post-colitis rat model [Ceuleers et al. Neurogastro 2015, DDW 2016, DDW 2017]. The objective of this study was to explore which serine proteases are specifically involved based on the profiles of the inhibitors used targeting tryptase, matriptase, uPA, cathepsin G and kallikrein 2, 4, 8.

Methods: As previously described, male Sprague-Dawley rats were intrarectally instilled with TNBS (colitis) or 0.9% NaCl (control). Successively, colonoscopies were performed to document the acute colitis on day 3 and the complete healing of the mucosa in the post-colitis phase (day 10-18). Colon sampling of control, acute colitis and post-colitis rats was performed to measure the mRNA expression of the serine proteases described above by qPCR, as well as to quantify mast cell tryptase by immunohistochemistry. Next, fecal samples were collected at day 0 (control), day 3 (acute colitis) and the day of sacrifice (post-colitis) to determine general protease activity using an azocasein assay.

Results (Table 1): All TNBS rats developed acute colitis on day 3, while the post-inflammatory status was confirmed on the day of sacrifice for post-colitis rats. The qPCR experiments showed a significant downregulation of matriptase in the colon of rats with acute colitis compared to controls. In post-colitis rats, tryptase was significantly upregulated,

24 | Involvement of the serotonin pathway in ileal neuromotor dysfunction associated with TLR2 and TLR4 inhibition in juvenile mice

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Objective: Oxidized phospholipids (OxPAPC) inhibit TLR2- and TLR4-dependent signalling recognized to be involved in ensuring enteric nervous system (ENS) integrity. This study aimed to evaluate the effects of in vivo OxPAPC administration on ileal contractility and serotonin pathway in juvenile mice.

Methods: Male mice C57BL/6J (3±1 weeks old) were treated intraperitoneally with OxPAPC (1.5 μg/g body weight) or vehicle (CNTR), twice daily for 3 days. Distribution of neuronal HuC/D and glial GFAP markers was determined by confocal immunofluorescence in longitudinal muscle-myenteric plexus preparations (LMMPs). Serotonin receptors (5-HTR) and SERT expression was evaluated by qRT-PCR and confocal microscopy in LMMPs. Plasma levels of 5-HT, tryptophan, 5-hydroxytryptophan and kynurenine were measured by HPLC-fluorescence detection. Contractile activity of ileum segments longitudinally mounted in organ baths was evaluated as changes in isometric muscle tension following electric field stimulation (EFS, 0-40 Hz) or 5-HT addition (0.01-10 μmol/L) with or without 0.1 μmol/L ketanserin (5-HT2AR antagonist) or 1 μmol/L ondansetron (5-HT3R antagonist).

Results: In OxPAPC myenteric plexus a significant reduction of HuC/D+ neurons and an increase of GFAP immunofluorescence were observed together with an altered 5-HTRs and SERT immunoreactivity. OxPAPC treatment increased 5-HT-mediated response of ileal segments by 1.4-fold and augmented mRNA levels of 5-HT2AR and SERT in LMMPs. EFS-evoked response was significantly higher in OxPAPC mice (Emax=+39±5.5%) and resulted differently affected by 5-HT2AR or 5-HT3R inhibition compared to CNTR mice. Interestingly, 5-HT plasma concentrations were below detection limit in OxPAPC mice. No differences in tryptophan and 5-hydroxytryptophan plasma levels were found whereas kynurenine and kynurenine/tryptophan ratio were significantly higher in OxPAPC compared to CNTR mice.

Conclusion: Our study demonstrated that OxPAPC-mediated TLR2 and TLR4 inhibition affects gut neuromuscular function and serotonin pathway during adolescence, suggesting an involvement of innate immunity in tryptophan metabolism and potentially in the microbiota-gut-brain axis.

Policy of full disclosure: None.

NEW TECHNOLOGIES IN CLINICAL NEUROGASTROENTEROLOGY

25 | Optogenetic induction of propagating colonic motor complexes and propulsion of fecal content induced by light

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Optogenetics is an exciting technique that has been shown to selectively control neural pathways in the central nervous system (CNS), but not in the enteric nervous system (ENS), leading to changes in gastrointestinal (GI) transit. The ability to selectively control GI-motility and GI-transit without using non-specific agonists or antagonists (that act all throughout the body) offers great hope for patients with impaired GI-transit, but without having to endure the side effects of non-specific drugs. Our aims were two fold. Firstly, to generate transgenic mice expressing channelrhodopsin in specific excitatory neurons of the enteric nervous system (ENS). Secondly, to demonstrate that specific wavelengths of light can modify colonic motility and the propulsion of content, without using any agonists or antagonists. To do this, we generated a novel transgenic mouse using Cre-driven expression of the light-gated cation channel, channelrhodopsin-H134R (ChR2-H134R) in excitatory enteric neurons expressing calretinin (CAL). Immunohistochemical analysis of colonic myenteric neurons revealed 97% of the cholinergic CAL-immunoreactive neurons were selectively expressing ChR2(H134R)-eYFP+. Mechanical recordings were made from intact whole colons in vitro (n=7). Both CAL-ChR2(H134R) and wild-type mice generated ongoing propagating neurogenic colonic motor complexes (CMCs), with a mean interval 280±37 seconds (n=7). Focal illumination (1-5 Hz, 10-60 seconds) of blue light to the proximal, mid or distal colon evoked a significantly premature CMC in CAL-ChR2(H134R) mice (P=.006, N=7; Figure 1), but not in the wild-type littermates. Also, green light had no effect in CAL-ChR2(H134R) mice. Video imaging of colonic wall movements revealed that light-evoked CMCs caused propulsion of fecal pellets over significant lengths along the isolated whole mouse colon of CAL-ChR2(H134R) mice. Tetrodotoxin prevented optogenetic activation of CMCs (7/7 times tested, n=3). We provide the first demonstration that focal illumination of light to specific regions along the large intestine can evoke propagating neurogenic contractions (CMCs), leading to the propulsion of fecal content. Optogenetics offers great potential for controlling the excitability of the ENS and improving GI-transit, without using drugs that are well known to act on receptors all throughout the body; and without inducing numerous unwanted side effects, in many organ systems. Funded by NH&MRC #1067335 N.J.S and an NIH RO1 to H.Hu.

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