Antibiotic-induced dysbiosis affects the structure and activity of enteric nervous system in mouse small bowel during adolescence

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Background. Gut microbiota is essential for the development of the gastrointestinal (GI) system. Any changes of its composition in early life triggered by environmental factors or drugs may impact enteric nervous system (ENS) homeostasis potentially leading to the onset of functional GI disorders later in life (Brun et al., 2013; Kennedy et al., 2014; Kabouridis & Pachnis, 2015). The present study aimed to assess the role of gut microbiota dysbiosis on small bowel excitatory and inhibitory neuromuscular pathways during mouse adolescence.

Methods. Gut microbiota dysbiosis was performed on male C57Bl/6J mice (3±1 weeks old) by administering a broad-spectrum antibiotic cocktail (50 mg/kg vancomycin, 100 mg/kg neomycin, 100 mg/kg metronidazol and 100 mg/kg ampicillin; ABX group) or vehicle (tap water; CNTR group) twice a day for 14 days by oral gavage (Reikvam et al., 2011). GI transit was assessed in ABX and CNTR mice 30 minutes after administration by oral gavage of a non-absorbable fluorescent-labeled dextran probe. In isolated ileum segments, mounted longitudinally in organ baths, changes in muscle tension were recorded by isometric transducers following electric field stimulation (EFS, 0-40 Hz) or EFS 10 Hz with or without 1 µM guanethidine + 1 µM atropine (NANC conditions), 100 µM Nω-nitro-L-arginine methyl ester (L-NAME, a pan-NOS inhibitor) or 10 µM L732138 (NK1 receptor antagonist). In ileal longitudinal muscle myenteric plexus preparations (LMMPs), the distribution of glial markers such as glial fibrillary acidic protein (GFAP) and S100β, and of neuronal markers such as HuC/D, neuronal nitric oxide synthase (nNOS) and substance P was analyzed by confocal immunofluorescence.

Results. In vitro contractility studies showed a significant reduction of neuronal cholinergic transmission (-60±7% at 10 Hz) in ABX mice, associated with an increase of tachykininergic-mediated response in NANC conditions in presence of L-NAME, that was abolished with pretreatment with NK1R antagonist. Moreover, in ABX mice changes in NANC relaxant response were found and were partially abolished by L-NAME. Antibiotic treatment determined slow GI transit and decreased number/hour output of fecal pellets. In the myenteric plexus of ABX mice S100β immunoreactivity was significantly increased (+40±5%) and was associated with altered density of GFAP+ gliofilaments. Changes in ENS neurochemical coding were evidenced by the reduction of HuC/D+nNOS+ (-35±4%) neurons together with an increased number of HuC/D+SP+ neuronal cells (+31±5%) in LMMPs of ABX mice compared to WT mice.

Conclusion. Our findings support the current view of a symbiotic relationship between microbiota and ENS highlighting the essential role of microbial products in fine tuning the development of the ENS, consequently contributing to preserve gut homeostasis and promote healthy aging (Hyland & Cryan, 2016).

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