Gut Microbiota Depletion Alters the Structure and Function of the Enteric Nervous System in Adolescent Mice

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Gut microbiota colonization has a key role during the development of enteric nervous system (ENS) circuitries (Collins et al., 2012; Kabouridis & Pachnis, 2015). Any change of its composition triggered by environmental factors or drugs may impact ENS homeostasis and determine the onset of functional bowel disorders (Brun et al., 2013; Saulnier et al., 2013). The aim of the present study was to evaluate the effect of gut microbiota depletion on ENS integrity and intestinal motility during mouse adolescence. Depletion of gut microbiota was performed on male C57Bl/6 mice (age=21±5 days) by administering for 14 days an antibiotic cocktail (50 mg/kg vancomycin, 100 mg/kg neomycin, 100 mg/kg metronidazol and 100 mg/kg ampicillin) by oral gavage every 12 hours (Reikvam et al., 2011). In antibiotic–treated (ABX) and control (CNTR) animals we assessed: i) gastrointestinal transit 30 minutes after intragastric administration of nonabsorbable fluorescein isothiocyanate labeled dextran; ii) pellet frequency, measured as changes in stool output during a 60 minutes collection period; iii) stool water content; iv) contractile activity of isolated ileum segments, vertically mounted in organ baths, measured as changes in isometric muscle tension following carbachol (0.001-100 μM), KCl (60 mM), electric field stimulation (EFS, 1-50 Hz) or inhibition in non-adrenergic, non-cholinergic (NANC) conditions (EFS=10 Hz, 1 μM atropine, 1 μM guanethidine, in the absence or presence of 0.1 mM L-NAME); v) distribution of the neuronal HuC/D and glial fibrillary acidic protein (GFAP) by double labelling confocal immunofluorescence in longitudinal muscle myenteric plexus preparations (LMMPs); vi) neurochemical coding integrity, evaluated by acetylcholinesterase biochemical staining, neuronal nitric oxide synthase (nNOS) immunohistochemistry and GluN1 mRNA levels in LMMPs. Antibiotic depletion determined a marked enlargement (+92±0.1%, $p<0.01$) in cecum, associated with a threefold increase in organ weight. ABX mice showed a significant reduction in the number/hour output of fecal pellets (-29.5±0.5%, $p<0.01$) and increased stool water content (+35±7%, $p<0.01$). Gastrointestinal transit was delayed in ABX mice compared to CNTR mice (GC 3.5±0.2 vs 7.3±0.2, respectively; $p<0.01$). In vitro contractility studies showed altered receptor- and KCl-mediated responses and an overall modified neuronal excitability. NANC-mediated relaxations were significantly increased (+144±30% at 10 Hz, $p<0.05$) in ileum segments from ABX mice. In the ENS of ABX mice HuC/D immunoreactivity decreased while acetylcholine-esterase$^+$ stained fibres increased (+37±3%, $p<0.01$). GFAP immunostaining showed distorted cellular processes as well as the distribution of nNOS$^+$ neurons was altered following gut microbiota depletion. A two-fold increase of GluN1 mRNA levels was also found in LMMPs of ABX mice. Gut microbiota depletion determines complex anomalies in ENS architecture, neurochemical coding and function leading to intestinal dysmotility. Altogether, such changes are highly indicative of the primary role played by the enteric microbiota in ensuring ENS integrity, consequently contributing to maintain the gut in a long-term healthy state.

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